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Original Articles

ANTIGENIC FRACTIONS IN RAGWEED POLLEN

I. WATER-SOLUBLE FRACTIONS

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THIS paper describes a method for the separation of two antigenic fractions from water extracts of low ragweed pollen, a study of the relative activity of these fractions both by direct and indirect skin tests, and a study of their ability to neutralize sensitive sera. It also deals with their antigenic relationship as determined by the Dale test. Further work is under way to separate and study other fractions from water extracts at different hydrogen ion concentrations, 10 per cent sodium chloride extracts, and ball mill extracts.

Caulfeild, Cohen, and Eadie[1] separated fractions from several pollens under varying conditions of extraction and precipitation, some of which probably involved protein denaturization. They noted differences of specificity both by scratch tests and the Dale tests. Bernton, Jones, and Csonka[2] did comparative scratch tests with fractions prepared from timothy pollen and concluded that the activity was not restricted to a single fraction. Johnson and Rappaport[3] prepared fractions from ragweed pollen by precipitation at varying concentrations of ammonium sulfate. Antisera were prepared in rabbits with these fractions, and by absorption of precipitins, it was concluded that there were at least two antigens in giant ragweed pollen. Stull, Cooke, and Chobot[4, 5] separated water-soluble protein fractions from giant ragweed and from timothy pollen. Since these separated proteins neutralized sensitive sera in passive transfer sites to test with the respective whole extracts, it was concluded that these were the active fractions of the pollens. Benjamins, van Dishoeck, and German[6] observed that slightly active ultrafiltrates from timothy pollen were rendered more active in skin tests when mixed with protein substances of greater molecular weight. Caulfeild, Brown, and Waters[7] prepared numerous fractions from rag-

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weed pollen, and, by neutralization of sera against whole extract in passive transfer, they concluded that "there is more than one allergically active substance in ragweed pollen solution, and, further, that in the serum of the ragweed hay fever individual there is more than one specific reagin." In a preliminary report, Hecht, Rappaport, and Welker[8] described fractions of ragweed pollen prepared by acidification and precipitation at various hydrogen ion concentrations. Filtrates after precipitation by heating to 60° C. at a pH of 4 gave little or no precipitation with antiragweed rabbit serum, but gave practically undiminished skin reactions by scratch test when compared with the original untreated extract of equivalent nitrogen content. Abramson, Sookne, and Moyer[9] showed that a protein-like material was adsorbed on quartz particles from giant ragweed pollen extracts and had an isoelectric point of pH 3.9. The adsorption of this material rendered the extract comparatively inactive on direct skin test.

SEPARATION OF TWO ANTIGENIC FRACTIONS FROM LOW RAGWEED POLLEN

For the purposes of the present study, 100 Gm. of ethyl ether defatted low ragweed (*Ambrosia elatior*) pollen was extracted at 7° C. for twenty-four hours with 1,000 c.c. of distilled water, and the pollen was filtered off. The pH of the extract which was 5.9 was adjusted by the addition of N/10 hydrochloric acid to pH 4 which was the approximate isoelectric point of the active fraction described by Abramson.[9] A small amount of insoluble material which separated was removed by centrifugalization and discarded. Precipitation was carried out at 50 per cent saturation with ammonium sulfate. This precipitated material was removed by centrifugalization and designated Fraction 1. The supernatant fluid was filtered, and, on saturation with ammonium sulfate, gave more precipitate, which was designated Fraction 2. These two fractions were reprecipitated ten times by dissolving in water, adjusting the pH to 4, when Fraction 1 was precipitated at 50 per cent saturation with ammonium sulfate and removed and Fraction 2 was precipitated at saturation. A certain amount of brownish material which failed to go into solution was discarded from each fraction up to the tenth reprecipitation when it did not appear. The two fractions were dialyzed against running water and then against distilled water until free from sulfate.

In order to isolate the solid material, 50 c.c. portions were dried from the frozen state in a cryochem apparatus. The microchemical analyses were carried out after further drying the samples in a vacuum at 100° C. Particular attention is drawn to the great difference in nitrogen content of these two dried fractions (Table I). To the remainder of the solutions, which were to be used for chemical and biologic tests, sodium chloride to 0.9 per cent concentration was added, when they were passed through a Seitz filter and cultured to prove sterility.

TABLE I

COMPARATIVE ANALYSIS OF DRIED FRACTION 1 AND FRACTION 2

	FRACTION 1	FRACTION 2
Color	Colorless	Brown
Ash	4.53%	5.30%
Nitrogen (Micro Dumas)	11.30%	1.68%
Nitrogen (Calculated on ash-free basis)	11.83%	1.75%
Phosphorus	0	0

TABLE II

COMPARISON OF PROPERTIES OF FRACTION 1 AND FRACTION 2

	FRACTION 1	FRACTION 2
Color	Colorless	Greenish brown
Volume	110 c.c.	160 c.c.
Total nitrogen (mg. per c.c.)	0.20	0.15
Protein nitrogen (phosphotungstic acid) (mg. per c.c.)	0.20	0.15
Protein nitrogen (trichloracetic acid) (mg. per c.c.)	0.10	No precipitate
Boiling	Precipitate	Clear
Alcohol (10 times volume)	Precipitate	Cloudy suspension
0.5 % alum	Precipitate	Cloudy suspension
Xanthoproteic test	Negative	Negative
Millon's test	Reddish precipitate	Yellow-red precipitate
P-dimethylamino-benzaldehyde test	Bluish precipitate	Negative
Biuret test	Pinkish purple	Obscured by color of solution
Molisch's test	Negative	Positive
Reduction of Benedict's solution (after hydrolysis)	None	Slight
Liquefaction of 15% gelatin	Positive	Negative
Tests for proteoses with nitric acid, mercuric chloride, or glacial acetic acid and potassium ferrocyanide	Negative	Negative

The two fractions showed marked differences in precipitation by heat and by chemical agents, in color tests for protein and carbohydrate, and in enzymatic[12] activity (Table II). Fraction 1 was characteristic of protein in that it contained nearly 12 per cent nitrogen, was nondialyzable through semipermeable membranes, was precipitated by phosphotungstic acid and by ammonium sulfate, and was antigenic as will be shown by the Dale tests. Fraction 2, although containing less than 2 per cent nitrogen, was also nondialyzable, precipitable by phosphotungstic acid and ammonium sulfate, and was antigenic.

REACTIONS OF THE FRACTIONS ON DIRECT SKIN TESTS ON SENSITIVE PERSONS

Fraction 1, Fraction 2, and whole low ragweed pollen extract (this latter was prepared by extraction of the defatted pollen in 0.5 per cent NaCl and 0.3 per cent sodium bicarbonate), were diluted on the basis of the protein nitrogen unit[10] (1 unit = 0.00001 mg. protein nitrogen) for comparison in intracutaneous and biologic tests.

TABLE III

REACTION OF FRACTIONS IN DIRECT SKIN TESTS ON HITHERTO UNTREATED CASES OF RAGWEED HAY FEVER

CASE	FRACTION 1 (UNITS PER C.C.)			FRACTION 2 (UNITS PER C.C.)			WHOLE EXTRACT (UNITS PER C.C.)		
	1	10	100	1	10	100	1	10	100
Ror.	+	++	+++	±	+	+	+	++	++
Heh.	+	+	++	+	++	+++	±	+	+++
Ges.	++	++	+++	±	+	+	+	++	++
Coe.	++	++	+++	0	+	++	++	++	+++
Anm.	+	+	+++	+	+	++	+	+	+++
Kar.	+	++	+++	+	++	+++	+	++	+++
Con.	+	+	++	+	+	++	+	+	++
Mia.	+	++	+++	0	0	+	+	+	+++
Wen.	++	++	+++	+	++	++	++	++	+++
Han.	+	+	+++	0	0	+	+	++	+++
Sal.	++	++	+++	±	+	+	++	+++	+++
Gre.	+	++	+++	+	+	+	+	++	+++
Rth.	+	++	+++	0	++	++	+	++	+++
Ans.	+	++	+++	+	+	++	+	++	+++
Lil.		++	+++		+++	+++		++	+++
Bum.	+	++	+++	±	+	++	+	++	+++
Nez.	+	++	+++	+	++	+++	+	++	+++
Bar.		+++			+			+++	
Kan.	+	++	+++	0	0	+	+	+	+++
Sil.		++			+			++	
Gry.		+	++		+	++		+	++
Stk.	+	+	+++	+	+	++	+	+	++
Spe.	+	++	+++	+	++	+++	+	++	+++

Skin tests were carried out on typical untreated cases of ragweed hay fever by the intracutaneous injection of 0.01 and 0.02 c.c. of three dilutions of each fraction and the whole extract. As shown in Table III, there was a definite variation in activity. In some cases the two fractions gave equal reactions, similar to those given by whole extract. In many cases the reaction to Fraction 2 was less than that to Fraction 1; in a few cases Fraction 2 gave greater reactions than Fraction 1.

REACTIONS OF THE FRACTIONS ON SENSITIZED SKIN SITES

The differences just mentioned above were more clearly shown by the indirect method of testing sites made with ragweed-sensitive serum. Triplicate sites were prepared in the backs of normal test subjects (persons who gave negative skin tests to ragweed extract) by the intracutaneous injection of 0.1 c.c. of a 1:10 dilution of serum from an untreated ragweed-sensitive patient. Forty-eight hours later these sites were tested with 0.025 c.c. of dilutions of Fraction 1, Fraction 2, and whole extract (Table IV). Dilutions of 1 unit per cubic centimeter were used in testing eleven sera, and dilutions of 10 units per cubic centimeter were used to test six other sera. In every case Fraction 1 gave as great or greater reactions than the whole extract; in seven sera, Fraction 2 gave no reaction; and in three others, Fraction 2 gave less than Fraction 1. In one serum (Heh.), Fraction 2 gave slightly greater reactions than Fraction 1 or whole extract.

TABLE IV

REACTION OF THE FRACTIONS ON PASSIVELY SENSITIZED SKIN SITES

RAGWEED-SENSITIVE SERA, 1:10	DILUTION OF TEST SOLUTION (UNITS PER C.C.)	REACTION OF TRIPLICATE SITES ON TEST		
		FRACTION 1	FRACTION 2	WHOLE EXTRACT
Heh.	1	++	+++	++
Ror.	1	+++	0	++
Mat.	1	++	+	++
Rat.	1	++	±	++
Ahn.	1	+	0	+
Sil.	1	+	0	+
Hes.	1	+	+	+
Orn.	1	+	+	±
Bra.	1	+	0	0
Hch.	1	+	0	+
Ben.	1	+	+	+
His.	10	+++	+++	+++
Axd.	10	+++	+	+++
Bru.	10	++	++	++
Meo.	10	++	++	++
Den.	10	++	0	++
Hit.	10	++	0	++

With the serum Sil., 1,000 units of Fraction 2 were required to produce a positive reaction equal to that produced by 1 unit of Fraction 1.

COMPARATIVE NEUTRALIZATION OF SENSITIVE SERA BY THE FRACTIONS AND WHOLE EXTRACT OF LOW RAGWEED

Neutralization of sensitive sera in passive transfer sites seems to furnish a more delicate means of determining differences in specificity and activity of pollen extracts than direct or indirect skin tests. The ragweed-sensitive sera were obtained from patients who had never received injections of pollen extract. Serum was mixed in vitro with equal volumes of the two fractions or whole extract in varying strengths, according to the quantitative technique previously used in this clinic.[11, 12] Triplicate sites were made with each of these mixtures by injecting 0.1 c.c. intracutaneously in each of three areas in the back of a healthy nonallergic test subject who gave a negative skin reaction to ragweed extract. The sites so made were marked, and forty-eight hours later the triplicate sites of each mixture were tested with 0.025 c.c. of 1,000 unit dilution of Fraction 1, Fraction 2, and whole extract, respectively.

Table V shows typical examples of the comparative neutralization of eight sensitive sera with the fractions and whole extract. In all cases (except that serum Heh. in 1:10 dilution was mixed with Fraction 1 and Fraction 2, but not with whole extract), 1,000 units of whole extract neutralized to test with both fractions as well as with whole extract. In all cases, Fraction 1 in 1,000 units neutralized to test with itself. In several cases it also neutralized to test with Fraction 2 or whole extract. In other cases (serum Kwr. and serum Heh.), Fraction 1 did not neutralize to Fraction 2 or whole extract. In another serum

TABLE V

COMPARATIVE NEUTRALIZATION OF SENSITIVE SERA WITH LOW RAGWEED FRACTIONS AND WHOLE EXTRACT

SERUM	MIXED WITH	PROTEIN NITROGEN UNITS	TRIPLICATE SITES REACTION ON TEST WITH 1000 UNITS PER C.C.		
			FRACTION 1	FRACTION 2	WHOLE EXTRACT
Rat.	Fraction 1	1,000	0	0	+
	Fraction 2	1,000	++++	+	++++
	Fraction 2	10,000	++	0	++
	Whole extract	1,000	0	0	0
	Saline		++++	++	++++
Kwr.	Fraction 1	1,000	0	+	+
	Fraction 2	1,000	++	±	+++
	Fraction 2	10,000	++	0	++
	Whole extract	1,000	0	0	0
	Saline		++	++	+++
Ben.	Fraction 1	100	±	±	+++
	Fraction 1	1,000	0	0	+++
	Fraction 2	1,000	+++	+	++++
	Fraction 2	10,000	++	0	+++
	Whole extract	100	+	0	+
	Whole extract	1,000	0	0	0
	Saline		++++	++	++++
Sil.	Fraction 1	1,000	0	0	+
	Fraction 2	1,000	++	±	+++
	Fraction 2	10,000	+	0	±
	Whole extract	1,000	0	0	0
	Saline		+++	++	+++
Frn.	Fraction 1	1,000	0	0	+
	Fraction 2	1,000	++	+	++
	Fraction 2	10,000	0	0	0
	Whole extract	1,000	0	0	0
	Saline		+++	++	+++
Hor.	Fraction 1	1,000	0	0	±
	Fraction 2	1,000	+++	++	+++
	Fraction 2	10,000	+	0	++
	Whole extract	1,000	0	0	0
	Saline		++++	+++	++++
Jod.	Fraction 1	1,000	0	+	++
	Fraction 2	1,000	+++	+	+++
	Fraction 2	10,000	++	0	++
	Whole extract	1,000	0	0	0
	Saline		+++	++	+++
Heh. 1:10	Fraction 1	100	0	++	+
	Fraction 2	100	++	++	++
	Saline		+++	+++	+++

(Ben.), Fraction 1 neutralized to both Fraction 1 and Fraction 2 but not to test with whole extract, suggesting that this serum had sensitivity to other antigenic factors in the whole extract. Fraction 2 was relatively less active in neutralization, usually requiring 10,000 units to neutralize to itself, and it only neutralized to whole extract or Fraction 1 in occasional sera (not shown in table).

Heating to 70° C. for one hour largely destroyed the serum-neutralizing capacity of both Fraction 1 and Fraction 2, as it did of whole extract.[12]

ANTIGENIC RELATIONSHIP OF FRACTION 1 AND FRACTION 2 AND WHOLE EXTRACT AS DETERMINED BY THE DALE TEST

Virgin guinea pigs were sensitized by a single intra-abdominal injection of 50,000 units of alum-precipitated Fraction 1, or Fraction 2, or whole low ragweed extract. After a two- to three-week interval, the Dale test was performed with the sensitized uterine horns, using the technique previously described.[13] The fractions or whole extract in amounts of 10,000 units were added to the bath containing the uterine horns. If a contraction resulted, the same preparation was added until desensitization was obtained, before another preparation was tested. For brevity, these subsequent additions to prove desensitization, as well as the histamine contractions, are not indicated in Table VI.

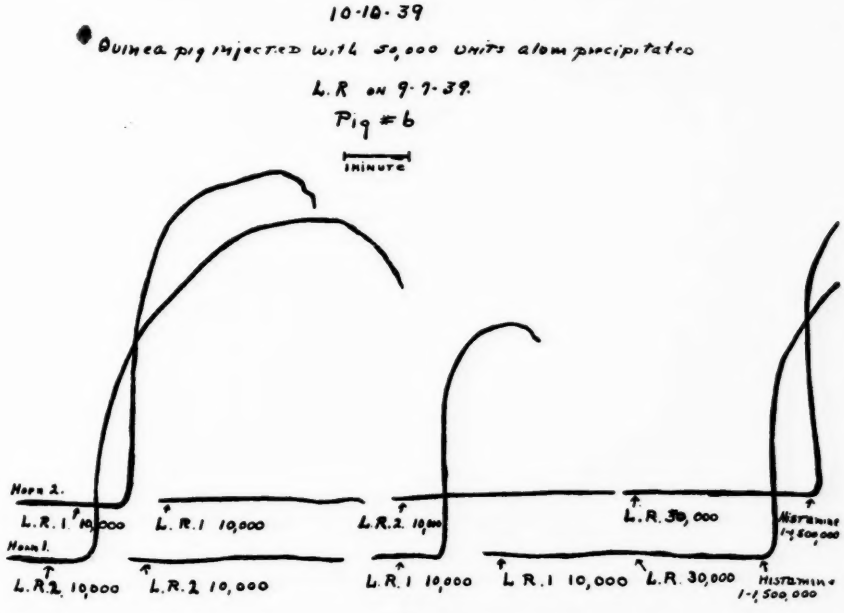

Fig. 1.

As shown in Table VI, in six of the seven pigs (Nos. 6-12) sensitized with whole extract, Fraction 2 produced a contraction of the first horn which again contracted on subsequent addition of Fraction 1. This horn was then desensitized to whole extract. Fraction 1 on the second horn produced a contraction and desensitized to both Fraction 2 and whole extract (Fig. 1). Whole extract desensitized to both Fraction 1 and Fraction 2. One pig (No. 7) showed sensitivity only to Fraction 1.

The horns from pigs injected with Fraction 1 (Fig. 2) usually gave a contraction on test with Fraction 2, but this fraction never desensitized to test with Fraction 1 (the sensitizing antigen). Fraction 1 desensitized to Fraction 2 and whole extract. Pig No. 1 showed sensitivity only to Fraction 1.

TABLE VI

SUMMARY OF DALE TESTS

PIG NO.	SENSITIZING ANTIGEN	HORN NUMBER	PREPARATION ADDED	CONTRACTION
6 (Fig. 1)	Whole extract	1	Fraction 2	+
			Fraction 1	+
			Whole extract	0
		2	Fraction 1	+
			Fraction 2	0
			Whole extract	0
7	Whole extract	1	Fraction 1	+
			Fraction 2	0
			Whole extract	0
		2	Fraction 2	0
			Fraction 1	+
			Whole extract	0
8	Whole extract	1	Fraction 2	+
			Fraction 1	+
			Whole extract	0
		2	Fraction 1	+
			Fraction 2	0
			Whole extract	0
9	Whole extract	1	Fraction 2	+
			Fraction 1	+
			Whole extract	0
		2	Whole extract	+
			Fraction 1	0
			Fraction 2	0
10	Whole extract	1	Fraction 2	+
			Fraction 1	+
			Whole extract	0
		2	Whole extract	+
			Fraction 1	0
			Fraction 2	0
11	Whole extract	1	Fraction 2 (heated 70° C.)	+
			Fraction 1 (heated 70° C.)	0
			Fraction 1	+
			Whole extract	0
		2	Fraction 2	+
			Fraction 1	+
			Whole extract	0
12	Whole extract	1	Fraction 2 (heated 70° C.)	+
			Fraction 2	0
			Fraction 1	+
			Whole extract	0
		2	Fraction 1 (heated 70° C.)	±
			Fraction 1 (heated 70° C.)	0
			Fraction 2	+
			Fraction 1	+
			Whole extract	0
3 (Fig. 2)	Fraction 1	1	Fraction 2	+
			Fraction 1	+
		2	Fraction 2	+
			Fraction 1	+

TABLE VI—CONT'D

PIG NO.	SENSITIZING ANTIGEN	HORN NUMBER	PREPARATION ADDED	CONTRACTION
3A	Fraction 1	1	Fraction 2	+
			Fraction 1	+
			Whole extract	0
		2	Whole extract	+
			Fraction 1	0
			Fraction 2	0
2	Fraction 1	1	Fraction 2	+
			Fraction 1	+
		2	Fraction 1	+
			Fraction 2	0
1	Fraction 1	1	Did not react	
		2	Fraction 2	0
			Fraction 1	+
			Whole extract	0
4	Fraction 2	1	Fraction 2	+
			Fraction 1	0
		2	Fraction 1	+
			Fraction 2	0
5 (Fig. 3)	Fraction 2	1	Fraction 1	0
			Fraction 2	+
		2	Fraction 1	0
			Fraction 2	+
5A	Fraction 2	1	Fraction 1	0
			Fraction 2	+
		2	Fraction 1	0
			Fraction 2	+

The horns from two of the three pigs sensitized with Fraction 2 reacted to Fraction 2 but not to Fraction 1 (Fig. 3). In the pig (No. 4) reacting to both Fraction 1 and Fraction 2, there was cross desensitization.

When Fraction 1 was heated to 70° C. for one hour, it was practically inactive in producing a Dale contraction and did not desensitize to the unheated Fraction 1 (pigs Nos. 11 and 12). Similar treatment of Fraction 2 did not destroy its desensitizing capacity for unheated Fraction 2 (pigs Nos. 11 and 12). It is of interest here to recall that, in the experiments cited before dealing with neutralization of human ragweed-sensitive sera, heating to 70° C. had practically destroyed the activity of both Fraction 1 and Fraction 2.

FRACTIONS OF GIANT RAGWEED

Fractions similar to Fraction 1 and Fraction 2 from low ragweed pollen have been separated from giant ragweed (*Ambrosia artemisiaefolia*) and showed specificities and antigenic relationships.

DISCUSSION

As a result of procedures used for the separation of fractions from whole low ragweed pollen, it would seem that, in the so-called Fraction 1,

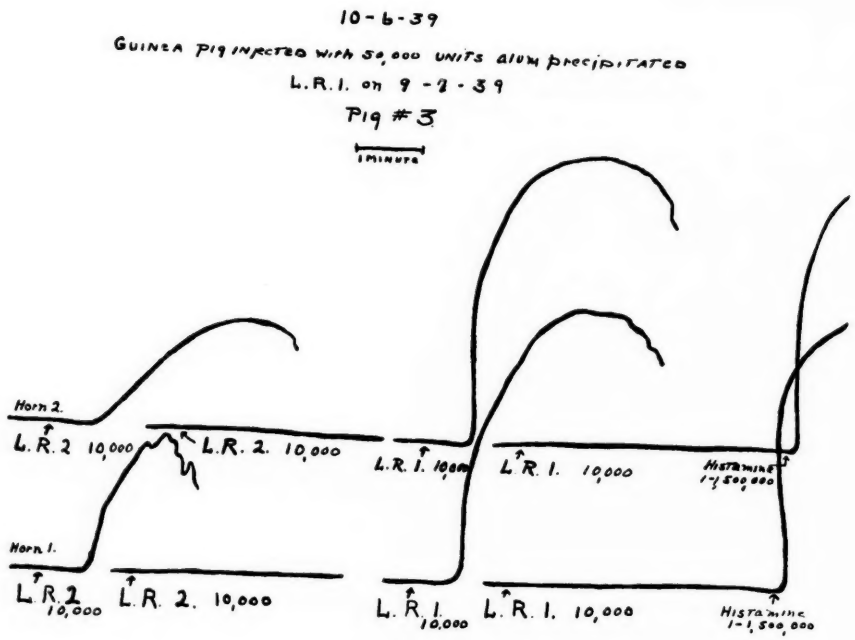
10-6-39
GUINEA PIG INJECTED WITH 50,000 UNITS ALUM PRECIPITATED
L.R.1. on 9-7-39
PIG #3
1 MINUTE
Horn 2.
L.R.2 10,000
L.R. 2. 10,000
L.R. 1. 10,000
L.R. 1. 10,000
HISTAMINE 1-1,500,000
Horn 1.
L.R.2 10,000
L.R. 2. 10,000
L.R.1. 10,000
L.R. 1. 10,000
HISTAMINE 1-1,500,000

Fig. 2.

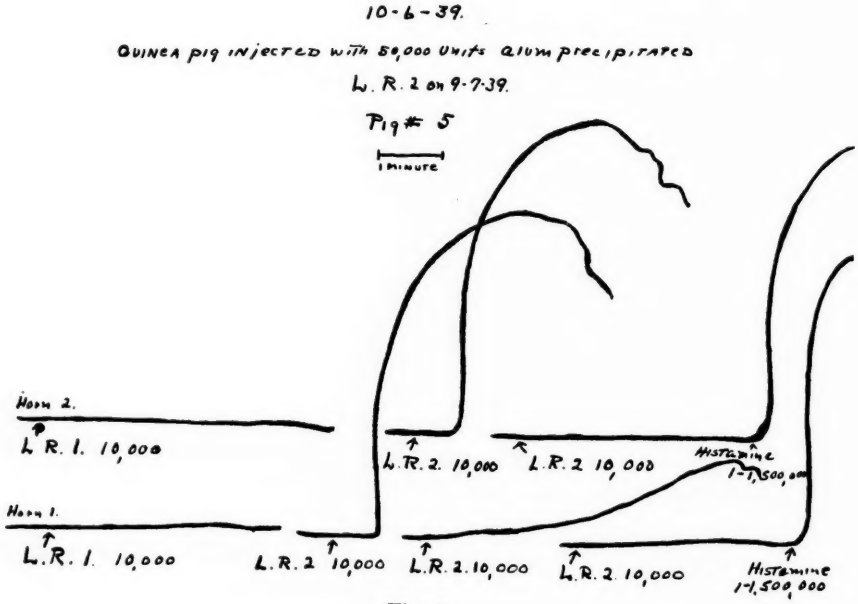
10-6-39.
GUINEA PIG INJECTED WITH 50,000 UNITS ALUM PRECIPITATED
L.R.2 ON 9-7-39.
PIG # 5
1 MINUTE
Horn 2.
L.R. 1. 10,000
L.R. 2. 10,000
L.R. 2 10,000
HISTAMINE 1-1,500,000
Horn 1.
L.R. 1. 10,000
L.R. 2 10,000
L.R. 2. 10,000
L.R. 2. 10,000
HISTAMINE 1-1,500,000

Fig. 3.

we have obtained a substance typical of protein both in physical and chemical properties and in nitrogen content. It did not contain carbohydrate as evidenced by negative tests. It also has all of the qualities necessary to fulfill the requirements as an active substance in ragweed pollen because it reacts in all clinically sensitive hay fever cases directly or in sites made with sera of such cases. It neutralized or greatly reduced the activity of six of eight sera against test with whole ragweed extract, and it was antigenic in guinea pigs and desensitized the uterine horns completely in all of seven guinea pigs sensitized to whole extract. This fraction appears the same as that previously separated.[4]

The low nitrogen content of Fraction 2 suggests that protein may be only a part of this fraction. It contained carbohydrate and coloring matter. The present study did not determine whether these compounds are linked to a complex protein molecule or not. Fraction 2 had antigenic properties, as shown by reactions with ragweed-sensitized cells in naturally sensitive man and in artificially sensitized guinea pigs.

There are definite indications of antigenic specificity of Fraction 2 as well as a relationship with Fraction 1. Evidence for independent specificity is indicated since two of three guinea pigs sensitized with Fraction 2 gave Dale reactions with that fraction but not with Fraction 1. Also, in the cross neutralization reactions with human sera, there were sera where each fraction neutralized specifically to test with itself, and the relative activity of the two fractions in direct and in indirect skin tests varied greatly in different sensitive patients. Such differences in activity seem to be explained best as due to independent antigenic factors and not simply to different amounts of the same antigen.

The antigenic relationship of the two fractions, however, is suggested by the Dale test on guinea pigs sensitized with whole extract where Fraction 1 invariably desensitized one horn to Fraction 2, while the second horn gave evidence of sensitivity to Fraction 2, and by the fact that cross neutralizations occurred also in some of the human sensitive sera.

Fraction 1 appears as the more active of these two antigens of ragweed pollen extract. However, the direct and indirect skin tests in many cases of hay fever indicate that Fraction 2 is an important factor, while in others there is evidence of very little sensitivity to this antigen. With sera of certain patients, the cross neutralization experiments indicated the presence of sensitivity to other antigens in the whole pollen extract not present in Fraction 1 or Fraction 2, and they are subject to further study.

The presence of two or perhaps more independent antigens in the pollen extract, the reactivity of which varies greatly in different ragweed-sensitive persons, complicates tremendously the standardization of pollen extracts. No method of standardization can express the activity of a whole pollen extract for all sensitive hay fever cases if some are equally sensitive to two or more antigens, while others react only to one.

It seems probable that the study of these active fractions in relationship to clinical hay fever and the use of them separately for serologic and clinical study may help to advance the knowledge of ragweed sensitivity and may aid in treatment.

SUMMARY

Two water-soluble fractions have been separated by fractional precipitation from low ragweed pollen extract. Each of these had certain characteristics of proteins, but showed marked differences in their nitrogen content and in chemical reactions.

The two fractions gave positive skin reactions in direct tests on ragweed-sensitive patients and on testing sites prepared with the serum of ragweed-sensitive patients (passive transfer). The relative activity of these two fractions varied greatly in different cases.

The two fractions also showed marked differences in their capacity to neutralize sensitive sera. Here again, their relative activities varied with different sera.

Studies by the Dale test showed that each of the two fractions had a certain antigenic specificity as well as cross relationship.

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ACQUIRED SPECIFIC HYPERSENSITIVITY TO SIMPLE CHEMICALS

VI. Further Studies on the Purification of Dyes in Relation to Allergic Reactions

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THE sensitizing effects of dyes and dye intermediates have been the subject of several previous communications of this series.[1-5] Among the results of these preceding studies, only those dealing with the effects of certain dyes in cosmetics, and particularly in lipsticks, are directly connected with the present experiments. The following are the pertinent findings which led to the present extension of our investigations:

1. Many cases of sensitivity to cosmetics are in some way attributable to a dye or to dyes contained in the respective preparations.

2. The sensitivity to these dyes is a specific and acquired one, i.e., one which is based upon allergic alterations. In our cases, the particular allergic reaction was almost always that of contact-type eczematous dermatitis.

3. Many cases of cheilitis (irritation and inflammation of the lips) in women are attributable to lipsticks, and particularly to the dyes in lipsticks.

4. Among the lipstick dyes, the indelible dyes—the tetrabromfluorescein series—were most commonly responsible for the allergic reactions; but other dyes or colors were also sensitizers and accounted for some of the reactions. (In our series, lithol red was second to tetrabromfluorescein in its sensitizing capacity.)

5. On patch testing with different commercial brands of these various dyes, it was found that the reactions were variable; different manufacturing lots of what were purported to be exactly the same dye sometimes produced different degrees of reaction even when applied simultaneously to symmetrical sites in the sensitive skin.[5]

6. It is known that the commercial brands of tetrabromfluorescein contain varying amounts of different kinds of impurities. Purification of these commercial dyes reduced, and sometimes even abolished, the capacity of the dye to produce the reactions in the allergic skin.[5]

In connection with this last point, Table I shows the patch test reactions in twelve patients with lipstick cheilitis and allergic sensitivity to commercial tetrabromfluorescein. Five of these cases were

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From the Skin and Cancer Unit, New York Post-Graduate Medical School, Columbia University, and from the Department of Dermatology of the University of Illinois.

TABLE I

TWELVE PATIENTS WITH CHEILITIS DUE TO LIPSTICK DYES AND WITH PATCH TEST REACTIONS TO COMMERCIAL BROMINATED FLUORESCEINS

	CALCO-BROMO BLUE SHADE COMMERCIAL†	CALCO-BROMO YELLOW SHADE COMMERCIAL‡	PURIFIED TETRA-BROMFLUORESCEIN§
N. O.*	+	+++	–
R. B.*	+	++	–
B. M.*	++	+	(+)
M. W.*	+++	++++	–
A. A.	++	++	–
M. J.	++	+++	–
J. G.*	++	++	–
M. G.	++	++	–
M. L.	++	++	–
L. S.	++	+++	–
O. S.	+++	+++	–
M. I.	++	++++	–

*Previously reported cases.

†Calco-bromo blue shade is commercial grade of tetrabromfluorescein without any purification. This is the quality used for lake purposes.

‡Calco-bromo yellow shade represents a commercial product of the quality used for lake purposes. It consists of a mixture of dibrom- and tetrabromfluoresceins without any purification.

§Purified tetrabromfluorescein is the best grade of commercial tetrabromfluorescein purified by passing through a soluble salt and back to the free acid, followed by crystallization of a soluble salt from alcohol and conversion to the free acid.

reported in our previous communications and seven are additional, new ones. Table I demonstrates that all these cases gave strong allergic reactions to the commercial grades of the dyes; and with one exception, all gave *negative* reactions to the *purified* dye. (The exception is B. M., who, however, gave only a very slight reaction to the purified dye.)

The following possible explanations were advanced to account for the findings that the *purified tetrabromfluorescein* no longer elicited the same degree or number of reactions as had the impure commercial varieties:

1. Perhaps the sensitivity studied is not usually directed to the tetrabromfluorescein itself, but is actually due to sensitization to one or more of the common accompanying impurities.

2. Perhaps neither the tetrabromfluorescein alone nor the impurities alone can elicit the skin reaction. Perhaps the *combined action of pure dye and of certain impurities,* or the synergistic action of the dye and of the impurities, is necessary to elicit the allergic response.

3. A third possibility must also be considered. Perhaps the various processes of purification employed have so altered the original pure dye that, although it seems to maintain its chemical identity, it has nevertheless been changed in regard to its biologic property of producing reaction in the allergically sensitive skin.

Obviously a further study of these various possibilities was indicated, for the results might be of fundamental theoretical significance in regard to the general problems of sensitization of eczematous type. Moreover, the results of further studies might be of practical significance in relation to the attempts to prevent clinical reactions to dyes

or their impurities, and in relation to the attempts to establish standards for purity in the manufacture of commercial dyes. These practical aspects are, of course, of particular and immediate importance in view of the present high incidence of allergy to dyes and dyed materials.

EXPERIMENTAL DATA

Experiment 1.—The first experiment was designed to ascertain whether the *purification processes employed destroyed the reaction-eliciting property of the commercial dyes or whether they removed the impurities.*

For this purpose we applied patch tests with both the purified dye and with the mixtures of dye impurities which were removed during the 5 steps of the purification process. The materials removed during the separate steps of purification were designated the "residues," respectively, Residue 1 (removed during the first step of purification) to Residue 5 (removed during the fifth step).

The original material was a commercial technical grade of tetrabromfluorescein such as is sold to lipstick manufacturers, and kindly supplied to us by the manufacturer who carried out the various steps of purification.* These consisted in repeated passing of the original material "through a soluble salt and back to the free acid, followed by crystallization of a soluble salt from alcohol and conversion to the free acid."

The first residue, i.e., the residue from the first crystallization, contains a large quantity of brominated fluoresceins, together with most of the impurities removed from the commercial dye. The second to fifth residues still contain large quantities of brominated fluoresceins, but progressively diminishing quantities of impurities.

All the dyes and residues employed in these studies were first tested on eleven control individuals, i.e., by patch testing eleven patients who had cheilitis but no allergic sensitivity to dyes. None of the materials elicited any reaction in these control subjects, demonstrating that the reactions elicited in the experimental series *were not due to irritating properties of the dyes or residues,* but were based on *cutaneous hypersensitivity to the specific substances.*

For the purpose of our experiment, we had available 8 patients with cheilitis due to lipstick dyes, of whom 7 showed skin hypersensitivity to tetrabromfluorescein as demonstrable by positive reactions to patch tests with the commercial available "impure" dye. Table II shows the results of simultaneous patch tests with commercial tetrabromfluorescein, with the first residue, with the third residue (containing the removed impurities), and with the purified dye, i.e., with the tetrabromfluorescein after removal of impurities by five separate purifications. It will be noted that, in these 8 cases of lipstick cheilitis due to dyes, 6 gave definite reactions to the commercial tetrabromfluorescein dye and one (M. I.) gave a questionable reaction. More important still, it will be seen that in 6 instances the reaction to the first residue was stronger

*We are indebted to Dr. David R. Climenko and to Dr. M. L. Crossley, of the Calco Chemical Company, for executing the chemical parts of these experiments, and for supplying us with the various commercial dyes and colors used in these studies.

than that to the commercial dye, and in the other 2 instances, equal to the reaction from the commercial dye (M. J. and M. W.). Furthermore, in all instances the reaction to the third residue was less than that to either the commercial dye or the first residue, and in the tests with the dye purified 5 times, the reaction was entirely abolished in 7 cases, and very materially diminished in one case (M. W.).

TABLE II

EIGHT PATIENTS WITH CHEILITIS DUE TO LIPSTICK DYES AND WITH PATCH TEST REACTIONS TO COMMERCIAL TETRABROMFLUORESCEIN OR ITS IMPURITIES

	TETRABROM-FLUORESCEIN COMMERCIAL*	FIRST RESIDUE†	THIRD RESIDUE‡	PURIFIED TETRABROM-FLUORESCEIN§
M. J.	++	++	−	−
A. A.	+	++	+	−
L. S.	+	++	−	−
M. W.	+++	+++	++	+
M. I.	?	±	−	−
B. M.	−	±	−	−
O. S.	++	+++	+	−
R. H.	++	+++	±	-

*Tetrabromfluorescein commercial is a technical grade of tetrabromfluorescein.

†First residue is the residue obtained from the first purification step. It contains a large quantity of brominated fluoresceins together with most of the impurities which were removed in the first purification step.

‡Third residue is the impurities obtained from the third purification step. As in the case of the first residue, this material contains large quantities of brominated fluoresceins but other impurities are present.

§Purified tetrabromfluorescein is purified by five separate purification steps. The above first and third residues are those obtained after the first and third purification steps.

These results indicate that the purification processes did not *destroy,* but rather *removed,* some allergenic ingredient or ingredients, some impurities responsible for the patch test reactions to the commercial dye. They further indicate that the ingredient or ingredients responsible were present in highest concentration in the first residue, in lesser concentration in the original impure dye, in still lower concentration in the third residue, and in least concentration in the "purified dye."

It may in the future be possible to identify by chemical studies the exact allergenic ingredient or ingredients responsible, to ascertain which substance or substances are present in the above-mentioned relative proportions in the original dye, in the residues, and in the purified tetrabromfluorescein.

Experiment 2.—Up to the present, the fact that the allergic contact-type skin reactions to dyes are not based upon the pure dye itself but upon the accompanying impurities, or upon the combined action of impurities and pure dye, has been established only in the case of tetrabromfluorescein. The theoretical and practical application of these findings would, of course, be considerably greater if it were discovered that this important role of impurities is not confined to tetrabromfluorescein alone, but is equally applicable to other dyes, colors, and substances.

Our previous studies[5] had suggested that the same laws might be applicable to lithol red as to tetrabromfluorescein, and that the allergic reactions to lithol red were also in some way due to the impurities present.

In the following experiment, we endeavored to discover whether the purification of lithol red and of dibromfluorescein reduced the capacity of the respective commercially available dyes to produce reaction in allergically sensitive skins.

Experiments With Lithol Red.—Patch tests with two commercially available salts of lithol red (impure) and the respective "purified" salts were applied simultaneously to 6 patients with cheilitis due to lipstick dyes.

TABLE III

SIX PATIENTS WITH CHEILITIS DUE TO LIPSTICK DYES

	LITHOL RED, SODIUM SALT, COMMERCIAL*	LITHOL RED, CALCIUM SALT, COMMERCIAL†	LITHOL RED, SODIUM SALT, PURIFIED‡	LITHOL RED, CALCIUM SALT, PURIFIED‡
O. S.	–	–	–	–
A. A.	–	–	–	–
M. I.	–	–	–	–
B. M.	±	±	–	–
M. W.	++	++	–	–
M. J.	±	–	–	–

*Lithol red, sodium salt, commercial is the usual grade of commercial product.

†Lithol red, calcium salt, commercial is the usual grade of commercial product.

‡Lithol red, sodium salt, purified and lithol red, calcium salt, purified are made by the regular laking method, using purified Tobias acid and C. P. beta-naphthol. The lakes were carefully washed to remove excess salts. We are indebted to the Calco Chemical Company for supplying and preparing these colors and the purified salts.

As shown in Table III, three of these six patients reacted to some degree to the commercial impure salts of lithol red. *There were no reactions to the purified salts of lithol red.*

While the number of experiments is too small to permit conclusions, these results seem to support our previous impression that the allergic patch test reactions to commercial lithol red are in some way dependent upon the impurities present, in a manner analogous to that demonstrated in relation to commercial tetrabromfluorescein and its impurities.

Dibromfluorescein.—Patch test studies with commercial dibromfluorescein (impure) and with "purified" dibromfluorescein were carried out in 10 patients with cheilitis due to lipstick dyes. As in all previous experiments, the commercial dye and the purified dye were applied simultaneously. Table IV shows the results of these tests. In 8 of the 10 cases, the reaction to the purified dye was much less than that to the impure commercial product. In 2 cases the reactions to commercial and purified dyes were equal (M. W. and J. G.). But in no instance was the reaction to the purified dye stronger than that to the commercial, impure form.

These results indicate that the allergic patch test reactions to commercial dibromfluorescein are in some way dependent upon the impurities

TABLE IV

TEN PATIENTS WITH CHEILITIS DUE TO LIPSTICK DYES AND WITH PATCH TEST REACTIONS TO COMMERCIAL DIBROMFLUORESCEIN

	DIBROMFLUORESCEIN COMMERCIAL*	DIBROMFLUORESCEIN PURIFIED†
N. O.	++	(+)
R. B.	++	–
B. M.	(+)	–
M. W.	+++	+++
A. A.	+	–
M. J.	+++	++
M. G.	++	+
J. G.	+++	+++
M. L.	+	–
L. S.	+++	–

*Dibromfluorescein commercial is commercial dibromfluorescein without purification. This is the technical grade produced for lake purposes.

†Dibromfluorescein purified is the commercial grade of dibromfluorescein purified by acid extraction.

present, in a manner analogous to that demonstrated in relation to commercial tetrabromfluorescein and its impurities, and perhaps also in relation to commercial lithol red and its impurities.

It may be of some significance to note that 2 of the strongest reactions (M. W. and J. G.) showed no diminution of reaction when tested with the purified dibromfluorescein. It seems possible that in these cases the skin sensitivity was of such high degree that the purified dye still contained enough impurities to produce the maximum responses. Other explanations are, of course, equally possible; for example, that unlike the other 8, these 2 particular patients were actually sensitive to the pure dye and not to the impurities.

DISCUSSION

Our present results confirm the original findings that the impurities present in certain commercial dyes and colors are in some way responsible for many of the allergic reactions to the commercial preparations. Certain measures were employed to remove these impurities. These measures did not *destroy* the reaction-eliciting properties of the dyes, but *removed the impurities directly or indirectly responsible for the allergic reactions.* The reaction-eliciting substances are present in various proportions in the residues removed by the purification process. In the case of tetrabromfluorescein, we have been able to demonstrate that purification by repeated precipitation is necessary to remove the reaction-eliciting substances and that each successive residue contains less and less of the responsible allergenic materials. It may be possible through quantitative analysis of these residues to ascertain what substance or substances or combinations are the ones responsible for the reactions. While it appears to us likely that the majority of allergic reactions to commercial tetrabromfluorescein are in some way connected with the presence of certain impurities, it is of course equally possible that at least some reactions may be due to the pure dye itself.

Our experiments with lithol red and with dibromfluorescein suggest that the finding that impurities are important factors in producing allergic reactions is not a peculiarity confined to allergic sensitivity to tetrabromfluorescein, but is probably of significance in relation to other dyes; it may even be more generally applicable to many different kinds of allergenic simple chemicals, and even to other types of allergens.[5]

As far as practical problems are concerned, it must still be ascertained whether it is feasible to manufacture dyes and colors used in commerce in such a way as to reduce the quantity of the impurities which may determine, or contribute toward, the common allergic reactions. Moreover, before engaging in changes in manufacturing processes, it will have to be ascertained whether the purer dyes will actually prove themselves substantially less allergenic under conditions of ordinary consumer use and clinical exposure. For, up to now, our results are based only upon the demonstrated lesser capacity of purified dyes to elicit reactions on skin tests in already sensitized allergic persons. Our present findings strongly suggest that the purer the dye, the less the likelihood of its producing allergic disease. Nevertheless, our results cannot be taken as proof that the relative sensitizing capacities of these substances will surely parallel their reaction-eliciting capacities, nor as proof that results of clinical exposures will entirely conform to the results of our patch test exposures.

To be specific, at present we are not yet certain that all of our patients with negative results to patch tests with purified dyes will actually tolerate lipsticks containing only the purified dyes; nor do we know how long they will tolerate the repeated application of such lipsticks to the lips; nor what the exposure to light and what many other factors may bring about when purified dyes replace the older forms of commercially available "impure" products.

Judgment will have to be deferred on all these points and on many other aspects of the problems which have been introduced by the present studies. Some of these problems certainly appear to be sufficiently important to deserve further investigations from both the theoretical and practical viewpoints, and to deserve attention from the chemical, immunologic, and biologic, the commercial and manufacturing aspects.

SUMMARY

1. Additional experiments are presented confirming the fact that patients with irritation of the lips due to lipstick (lipstick cheilitis) are often allergically sensitive to the commercial dyes used in these cosmetics.

2. Additional experiments are presented confirming the fact that "purification" of a commercial brand of tetrabromfluorescein abolished, or materially reduced, its capacity to elicit allergic reactions when applied as patch tests.

3. Experiments are presented demonstrating that the purification method employed, i.e., repeated crystallization of a soluble salt from alcohol, did not destroy the reaction-eliciting factors, but that the reaction-eliciting ingredients were removed by the purification processes.

4. It is shown that the reaction-eliciting ingredients are present in different proportions in the commercial dye and in the 5 residues removed by the 5 successive steps of purification. It is suggested that this finding may perhaps lead to the identification of the agent or agents or combinations responsible for the patch test reactions.

5. Experiments are presented indicating that the purification of commercial lithol red and of commercial dibromfluorescein reduced also their capacity to produce allergic reactions when applied as patch tests. This finding suggests that the demonstrated role of impurities, in being in some way responsible for allergic patch test reactions, may have more general applications and implications.

6. Some of the implications of these findings are discussed, and it is emphasized that further laboratory and clinical studies will be necessary to ascertain both the clinical and commercial applications of the present results and their wider theoretical significance.

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DISCUSSION

DR. JOSEPH HARKAVY, New York.—The title of this paper is rather misleading, because Dr. Sulzberger is not dealing, as he said, with "simple chemicals," but, on the contrary, with very complex substances. The significance of his paper lies in the fact that he re-emphasizes the complicated nature of antigens and allergens with which we are constantly working. We have, in the past, been rather smug in our acceptance of the fact that, when we get positive skin reactions to pollens or foods, we have a clear-cut demonstration of sensitivity to the particular food or pollen tested, but is it so? Dr. Sulzberger's observations negate our assumptions most impressively. It is important that more

of this type of work be carried out so that we may understand what fundamental factor is represented in a given positive skin reaction.

This is important not only to dermatologists but to all of us. For example, the recent work of Dr. Stull and his associates and also of Dr. Abramson of our department indicates that, when we deal with ragweed pollen, we are confronted with a very complex substance. Dr. Abramson, who is at present studying ragweed pollen by means of the Tiselius apparatus, told me that he has been able to demonstrate 6 different bands. It is the task of the future not only to identify these fractions but also to determine what relationship they bear to elements common in other substances which cause allergic manifestations. Dr. Sulzberger's work clearly indicates that "simple chemicals" are not so simple.

Dr. Bennett Kraft, Indianapolis.—I would like to ask Dr. Sulzberger and all the members of this group whether they have seen any untoward reactions from nylon. The nylon stockings have been on the market, at least in Indianapolis, for 6 weeks. By now there are 4 cases sensitive to it, all of them sick enough to be hospitalized. I wonder if it is that certain manufacturing houses have trouble with the dye, or if these are cases of sensitivity to nylon.

Dr. Sulzberger.—As Dr. Harkavy said, if such apparently simple substances as dyes can contain contaminants which we know nothing about and which are nevertheless the real causes of allergic reaction, certainly such complex allergenic mixtures, such veritable "soups" as tuberculin, trichophytin, milk, eggs, pollen extracts, and so forth, can contain many contaminating substances which may be the allergens in one case or another, and of which we have no idea as yet today.

In regard to what Dr. Kraft said about the nylon stockings, the answer is that I have seen and have heard of several such reactions. [N.B.: Since completing this discussion, Drs. Louis Schwartz, Louis W. Spolyar, Frank M. Gastineau, John E. Dalton, Adolph B. Loveman, Ellis P. Cope, Rudolf L. Baer, and I have studied this form of stocking dermatitis and have been able to demonstrate that the reactions in our cases were not due to nylon itself but to a chemical substance used as a "finish" in the manufacture of certain brands of the stockings. The detailed report of these findings appeared in the *Journal of the American Medical Association*, **115**: 906, 1940.]

Studies of biologic analysis and determination of the sensitizing indices of various combinations of the ingredients in nylon stockings, such as my associates and I have carried out with various other products, perhaps may be able to elucidate, and perhaps even to obviate, the sensitizing effects of such new material.

RESULTS OF SKIN TESTS FOLLOWING IMMUNIZATION AGAINST ENCEPHALOMYELITIS (EASTERN AND WESTERN TYPES) WITH BIVALENT VACCINES OF CHICK EMBRYO ORIGIN

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THE occurrence of encephalomyelitis among laboratory workers exposed to the living virus while engaged in the preparation of vaccines for the immunization of horses has drawn attention to the necessity of human vaccination. Beard, Beard, and Finkelstein[1] have recently reported the successful vaccination of human beings with an antigen prepared from chick embryo propagated virus. We too have vaccinated a group of sixty-nine normal persons with a bivalent vaccine prepared from chick embryo propagated virus and rapidly dehydrated by the lyophile process.[2] Although no untoward reactions were experienced following the repeated subcutaneous injection of this vaccine, we have been concerned with the possibility of inducing in these persons a specific sensitization to chick embryo protein. In order to determine whether this occurred, we performed skin tests on twenty members of this immunized group, and the findings form the basis for this report.

TABLE I

THREE LOTS OF VACCINE EMPLOYED IN VACCINATION

LOT NO.	TYPE AND TREATMENT	MG. NITROGEN PER C.C.
1	Eastern, 46831—filtered	0.56
	Western, 45660—filtered	0.73
2	Eastern, 46835—unfiltered	1.20
	Western, 50747—unfiltered	1.48
3	Eastern, 46835—unfiltered	1.20
	Western, 45664—unfiltered	0.84

The vaccine used for immunization was prepared from virus grown on twelve-day chick embryos. A 20 to 25 per cent suspension of the diseased tissue was treated with 0.4 per cent formalin, centrifugalized, and dehydrated by the lyophile process. Three lots of vaccine were employed (Table I).

The vaccines were prepared separately and lyophile-processed in 10 c.c. quantities. On the day of vaccination, one container of each type of vaccine was restored with 1 c.c. of distilled water (one-tenth

From the Medical-Research Division, Sharp and Dohme, Glenolden, Pa.

the original volume), and equal amounts of the two types were mixed. This mixture constituted the final bivalent vaccine, 2 c.c. of it thus being equivalent to 20 c.c. of a mixture of the original Eastern and Western vaccines.

Treatment.—Thirty-seven persons received a single course of treatment which consisted of two subcutaneous injections given a week apart. Thirty-two subjects received two such courses of treatment, given at an interval of from five to six months. The dose varied. Slight swelling and redness with some pain and tenderness at the site of injection occurred in a few cases. This reaction disappeared in twenty-four hours. Several subjects had a moderately severe local reaction that lasted for from two to three days. Four subjects had a generalized reaction only after the third dose. This consisted of aching and malaise which lasted twenty-four hours. These reactions did not occur after each injection and caused no disability.

The subjects who showed reactions were purposely included among the twenty selected for the study of skin sensitivity. The type of treatment administered and the reactions that followed are shown in Table II. Eleven persons were tested one year after the first course of treatment; one was tested four months after the first course of treatment; and eight were tested about five months after the second course of treatment.

Test for Sensitivity.—About 0.02 c.c. of testing solution was injected intracutaneously on the outer surface of the arm. Readings were made fifteen minutes and twenty-four hours later (Table III). Each patient was tested with four solutions prepared as follows:

Solution A.—Filtered Western vaccine diluted with Coca fluid to contain 0.1 mg. of protein nitrogen per cubic centimeter. (Total nitrogen, 0.18 mg. per cubic centimeter.)

Solution B.—1:10 dilution of solution A.

Solution C.—Unfiltered Western vaccine diluted to contain 0.1 mg. of protein nitrogen per cubic centimeter. (Total nitrogen, 0.16 mg. per cubic centimeter.)

Solution D.—1:10 dilution of solution C.

A preparation of chick embryo suspension that had been put through all the stages of vaccine production, except that it was not inoculated with virus, was used for the other solution.

Solution E.—Filtered chick embryo suspension diluted with Coca fluid to contain 0.1 mg. of protein nitrogen per cubic centimeter. (Total nitrogen, 0.13 mg. per cubic centimeter.)

Solution F.—1:10 dilution of solution E.

Solution G.—Unfiltered chick embryo suspension diluted to contain 0.1 mg. protein nitrogen per cubic centimeter. (Total nitrogen, 0.13 mg. per cubic centimeter.)

Solution H.—1:10 dilution of solution G.

TABLE II

IMMUNIZATION OF HUMAN SUBJECTS AGAINST ENCEPHALOMYELITIS (EASTERN AND WESTERN TYPES). BIVALENT LYOPHILE-PROCESSED VACCINE EMPLOYED (2 C.C. = 20 C.C. ORIGINAL VACCINE)

CASE	FIRST DOSE				SECOND DOSE				THIRD DOSE				FOURTH DOSE			
	DATE	AMOUNT (C.C.)	REACTION		DATE	AMOUNT (C.C.)	REACTION		DATE	AMOUNT (C.C.)	REACTION		DATE	AMOUNT (C.C.)	REACTION	
			LOCAL	SYSTEMIC			LOCAL	SYSTEMIC			LOCAL	SYSTEMIC			LOCAL	SYSTEMIC
	Lot 1				*Lot 2*				*Lot 2*							
72	5/ 8/39	0.15	None	None	5/15/39	0.15	None	None								
78	5/ 8/39	0.25	None	None	5/15/39	0.25	None	None								
82	5/ 8/39	0.15	Slight	None	5/15/39	0.15	Slight	None								
75	5/ 8/39	0.15	None	None	5/15/39	0.15	None	None								
81	5/ 8/39	0.10	None	None	5/15/39	0.10	None	None								
69	5/ 8/39	0.10	None	None	5/15/39	0.10	Slight	None								
68	5/ 8/39	0.25	None	None	5/15/39	0.25	Slight	None								
15	5/12/39	0.25	None	None	5/19/39	0.25	None	None	11/28/39	0.25	None	None	12/5/39	0.25	Moderate	None
52	6/30/39	0.25	Slight	None	7/ 7/39	0.25	None	None	11/29/39	0.25	Moderate	None	12/6/39	0.25	Slight	None
48	6/23/39	0.25	None	None	6/30/39	0.25	None	None	12/ 1/39	0.25	Slight	Slight	12/8/39	0.25	Slight	None
9	5/26/39	0.25	Slight	None	6/ 2/39	0.25	Slight	None	12/ 1/39	0.25	Moderate	Slight	12/8/39	0.25	None	None
7	5/11/39	0.25	None	None	5/19/39	0.25	None	None	11/28/39	0.25	Moderate	None	12/5/39	0.25	Slight	None
18	5/26/39	0.25	None	None	6/ 2/39	0.25	None	None	11/29/39	0.25	None	Slight	12/6/39	0.25	None	None
12	5/12/39	0.25	Slight	None	5/19/39	0.25	None	None	11/29/39	0.25	Slight	None	12/6/39	0.25	Slight	None
49	6/23/39	0.25	None	None	6/30/39	0.25	Slight	None	12/ 1/39	0.25	Moderate	Slight	12/8/39	0.25	None	None
3	5/12/39	0.25	Slight	None	5/19/39	0.25	Moderate	None								
	Lot 3															
77	5/ 8/39	0.25	None	None	5/15/39	0.25	Slight	None								
76	5/ 8/39	0.25	Slight	None	5/15/39	0.25	None	None								
67	5/ 8/39	0.25	None	None	5/15/39	0.25	Slight	None								
	Lot 2															
93	1/ 3/40	0.25	Moderate	None	1/10/40	0.25	None	None								

TABLE III

INTRACUTANEOUS SKIN TESTS ON SUBJECTS IMMUNIZED AGAINST ENCEPHALOMYELITIS

CASE	A SOLUTION		C SOLUTION		E SOLUTION		G SOLUTION		B SOLUTION		D SOLUTION		F SOLUTION		H SOLUTION	
	15 MIN.	24 HR.	15 MIN.	24 HR.	15 MIN.	24 HR.	15 MIN.	24 HR.	15 MIN.	24 HR.	15 MIN.	24 HR.	15 MIN.	24 HR.	15 MIN.	24 HR.
72									0	0	0	0	0	0	0	0
78									0	0	0	0	0	0	0	0
82									±	0	0	0	±	0	0	0
75									0	0	0	0	0	0	0	0
81									0	0	0	0	0	0	0	0
68									0	0	0	0	0	0	0	0
69									0	0	0	0	0	0	0	0
15									0	0	±	0	0	0	0	0
48									0	0	0	0	0	0	0	0
52									0	0	0	0	0	0	0	0
9									0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0								
18	±	0	±	0	±	0	±	0								
12	+	0	+	0	0	0	0	0								
49	0	0	0	0	0	0	0	0								
3	0	0	0	0	0	0	0	0								
76									0	0	±	0	±	0	±	0
77									0	0	0	0	0	0	0	0
67									±	0	0	0	0	0	±	0
93	0	0	0	0	0	0	0	0								

+ = slight positive.
± = doubtful.
0 = negative.

The Western vaccine had aged for one year in the cold vault before dilution. The chick embryo suspension was freshly prepared.

RESULTS

The results are shown in Table III. No patient showed any reaction at the end of twenty-four hours. The immediate reading showed that only one subject (No. 12) gave a slight positive (+) reaction[3] to the vaccine, both filtered and unfiltered, that contained 0.10 mg. of protein nitrogen per cubic centimeter. However, he was negative to corresponding dilutions of chick embryo suspensions. This patient's skin was highly reactive. He had received two courses of treatment with a slight local reaction after the first, third, and fourth injections, but none after the second dose of vaccine. One other subject gave a similar slight reaction (+) to the unfiltered chick embryo suspension that contained 0.01 mg. of protein nitrogen per cubic centimeter. A corresponding suspension of filtered chick embryo gave a doubtful reaction.

Doubtful immediate reactions occurred in four other persons. These reactions can be discarded, however, as without significance since the skin of these patients was of the irritable type and our reading of the reactions was a very close one. The rest of the subjects were completely negative.

SUMMARY

This study would suggest that no specific sensitization demonstrable by skin tests occurs following immunization with lyophile encephalomyelitis vaccine as used by us, and that whatever reactions occur following its injection are due to either a nonspecific protein effect or to the high salt content of the lyophilized antigen, or to both.

We welcome this opportunity to express our grateful thanks to Mr. S. D. Beard of the Lederle Laboratories, Inc., for supplying us with Eastern type vaccine in bulk.

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SENSITIVITY TO CASEIN IN INFANTILE ECZEMA CONFIRMED BY BIOLOGIC TITRATION OF THE TESTING EXTRACT

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PART 1

THE antigenic power of casein is not diminished by heat.[1, 2] Cow and goat casein are immunologically identical or almost identical.[3, 4, 5] The presence or absence of hypersensitivity to casein in milk-sensitive persons is, therefore, of considerable practical importance, for if it is present, heated cow's milk or goat's milk would not be indicated in dietetic treatment, as Anderson, Schloss, and Stuart[3] suggested in 1932.

It is certain that lactalbumin is immunologically far more important than casein. Little attention has been paid to the latter by allergists. Some have even doubted that hypersensitivity to casein exists. Inasmuch as it is originally in colloidal solution and is then coagulated in the stomach, while most of the lactalbumin is always in true solution, it is readily seen why there is more opportunity for sensitization to lactalbumin to develop. However, the first step in the digestion of casein is splitting into soluble paracasein and a peptonelike body.[6] Furthermore, it is well known that in the infant's stomach not all the casein is coagulated, and that which is not coagulated may possibly change from a colloidal to a true solution in the alkaline medium of the small intestine, as casein is readily soluble in weak alkali.

In cow's milk there is about 3 times as much casein as lactalbumin. With present methods of feeding, infants are given huge amounts of casein in relation to their weights, often over four times as much as if they were receiving woman's milk. When these facts are considered, it seems that in the infant, at any rate, there is plenty of opportunity for sensitization to occur, and it has been shown that casein is antigenic[7] (also see below), although not as strongly so as lactalbumin. Furthermore, it is certain that sensitivity to casein can exist in the infant with eczema.[8, 9]

It is very difficult, if not impossible, to obtain preparations of casein entirely free from lactalbumin, as Ratner and Gruehl[10] have so clearly pointed out. Casein exists in milk in colloidal suspension, and lactalbumin partly in colloidal and partly in true solution; when the casein

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is precipitated in the process of preparation, some of the lactalbumin is adsorbed by it. Results of skin tests with casein are not reliable in the presence of lactalbumin sensitivity unless it is known that the casein preparation used either contained no lactalbumin, or not enough to have given positive skin tests to lactalbumin in the concentration used.

As far as we know, no series of cases has been tested with a specially purified casein preparation. The specially purified casein that was used in the present investigations was prepared by the method of Van Slyke and Baker[11] at the Lederle Laboratories under the direction of Dr. Arthur Coca; the lactalbumin was prepared in the same laboratory by the method of Schloss.[12] The preparation of casein contained 0.089 mg. N per cubic centimeter (approximately a 1:2,000 dilution).*

In seventy-five infants with eczema who gave positive intracutaneous skin tests to casein or to lactalbumin or to both, results were as follows:

Group 1: 35 (46.6 per cent) reacted to both casein and lactalbumin.
Group 2: 35 (46.6 per cent) reacted to lactalbumin alone.
Group 3: 5 (6.7 per cent) reacted to casein alone.

The situation in Groups 2 and 3 is clear; in Group 1 it is not. The question arises: Are these bona fide casein reactions, or are they, in reality, lactalbumin reactions from traces of lactalbumin in the supposedly pure casein? There is reason to believe that they are bona fide casein reactions, because if they were not, one would not expect so many negative casein reactions (46.6 per cent) in the presence of lactalbumin sensitivity. Also, many of the positive casein reactions were as large as the lactalbumin reactions, which would not be the case if they were due to small amounts of lactalbumin in the casein. It was found, furthermore, that goat casein would give a positive reaction every time that cow casein did (thirty cases); goat lactalbumin will not always cross with cow lactalbumin,[13] so that in many cases, even if the goat casein had been grossly contaminated with lactalbumin, it would have made no difference in the authenticity of the casein reactions.

All this is presumptive evidence, but it is not proof, and so we turned to animal investigation to determine: (1) whether or not the casein contained lactalbumin; and (2) if it did contain lactalbumin, whether it contained enough to have given the positive skin reactions.

PART 2

This experiment is to determine whether the casein extracts were contaminated with lactalbumin.

METHOD

The same extracts were used in the animal experiments as in the clinical investigations. Dilutions of the original materials were made with

*This concentration was found not to cause nonspecific irritation in normal infants.

physiologic solution of sodium chloride without the addition of a preservative. In most instances both sensitizing and shocking doses were in 1 c.c. volume. Syringes and needles used for lactalbumin injections were never used for casein and vice versa. Sterile precautions were used throughout.

Two groups of albino guinea pigs weighing between 190 and 340 Gm. were actively sensitized to lactalbumin and casein, respectively, by the intraperitoneal injection of 1.5 mg. on alternate days for 3 doses. After an incubation period of seventeen or eighteen days, those pigs sensitized to lactalbumin were tested by the intravenous injection of 1.5 mg. casein, using the ear vein as the site of injection. From eleven to forty-nine minutes later the pigs were reinjected intravenously with 1.5 or 0.15 mg. lactalbumin. The second group of pigs, sensitized to casein, were first tested with lactalbumin and then reinjected with casein. The degree of anaphylactic shock was noted in each instance, and autopsies were performed on all pigs dying as a result of the injection. In every case the findings were those of typical anaphylactic death, namely, beating heart and distended lungs.

RESULTS

Reference to Table I shows the results in 10 pigs sensitized to lactalbumin. A test dose of 1.5 mg. casein intravenously in no instance produced any sign suggestive of an anaphylactic reaction. All 10 pigs, however, succumbed to typical anaphylactic death when reinjected with lactalbumin a few minutes later. Even the 2 pigs (Nos. 15 and 17) receiving only 0.15 mg. lactalbumin died promptly. This clearly demonstrates that lactalbumin is a potent antigen, but that there is insufficient lactalbumin in 1.5 mg. casein to produce shock.

TABLE I

PIGS SENSITIZED WITH 1.5 MG. LACTALBUMIN AT EACH OF THREE INTRAPERITONEAL DOSES AT TWO-DAY INTERVALS, FOLLOWED BY AN INCUBATION PERIOD OF 17 OR 18 DAYS

PIG NO.	DOSE OF CASEIN INTRAVENOUSLY (MG.)	DEGREE OF SHOCK	TIME INTERVAL IN MINUTES	DOSE OF LACTALBUMIN INTRAVENOUSLY (MG.)	DEGREE OF SHOCK
1	1.5	0	41	1.5	Dead 3½ min.
3	1.5	0	41	1.5	Dead 3 min.
7	1.5	0	43	1.5	Dead 3½ min.
9	1.5	0	49	1.5	Dead 4 min.
11	1.5	0	41	1.5	Dead 3 min.
13	1.5	0	16	1.5	Dead 2 min.
15	1.5	0	15	0.15	Dead 2 min.
17	1.5	0	15	0.15	Dead 3½ min.
7a	1.5	0	11	1.5	Dead 3 min.
9a	1.5	0	41	1.5	Dead 2 min.

In Table II are shown the results of the intravenous injection of 1.5 mg. lactalbumin in 8 pigs sensitized to casein. Two died a typical anaphylactic death, 4 showed varying degrees of shock, and only 2 failed

TABLE II

PIGS SENSITIZED WITH 1.5 MG. CASEIN AT EACH OF THREE INTRAPERITONEAL DOSES AT TWO-DAY INTERVALS, FOLLOWED BY INCUBATION PERIOD OF 17 OR 18 DAYS

PIG NO.	DOSE OF LACTALBUMIN (MG.)	DEGREE OF SHOCK	TIME INTERVAL IN MINUTES	DOSE OF CASEIN (MG.)	DEGREE OF SHOCK
2	1.5	++	46	1.5	+
6	1.5	0	46	1.5	+
10	1.5	+++	32	1.5	Dead 4½ min.
12	1.5	0	34	1.5	+
14	1.5	+++	19 hr.	1.5	0
16	1.5	Dead 4 min.			
6a	1.5	+++	33	1.5	Dead 18 min.
10a	1.5	Dead 3½ min.			

to respond. This was interpreted to indicate that the sensitizing doses of casein were sufficiently contaminated with lactalbumin to produce specific sensitivity to lactalbumin. When the 6 surviving pigs were reinjected with casein, 2 died in shock, 3 displayed mild symptoms, and one did not react.

DISCUSSION

It may be argued that the pigs sensitized to casein reacted to lactalbumin because the lactalbumin was contaminated with casein, instead of the reverse. This is unlikely for the following reasons: First, in the chemical separation of these substances, the casein is precipitated and should bring with it minute quantities of lactalbumin, leaving a pure lactalbumin solution above. Second, lactalbumin is a much more potent antigen than casein. In Table II it will be noted that only two of six pigs died when injected with 1.5 mg. casein, and it is therefore inconceivable that two of eight pigs should die from the amount of casein that might be contained in 1.5 mg. lactalbumin. Third, only very minute quantities of an antigen are necessary to sensitize a pig if this antigen is administered in divided doses intraperitoneally, and yet ten pigs sensitized to lactalbumin in this manner (Table I) failed to respond to casein. Had there been appreciable quantities of casein in the sensitizing lactalbumin doses, there should have been a response when the pigs were tested with casein.

From these results it is concluded that there is a detectable amount of lactalbumin in the casein extract.

PART 3

The purpose of this part of the experiment is to determine approximately how much lactalbumin is present as a contaminant in the casein. Since passive sensitization of guinea pigs produces a more uniform degree of sensitivity than active sensitization, this method was used for titrating the minimal lethal doses of lactalbumin and casein in pigs which, twenty-four hours earlier, had received 0.8 c.c. of rabbit antilactalbumin serum.

METHOD

The lactalbumin used for producing the antilactalbumin rabbit serum was produced in accordance with Coca's[14] directions. The lactalbumin and casein used for shocking were the same extracts as those used in the clinical work.

Several rabbits were given repeated intraperitoneal and intravenous injections of 10 mg. lactalbumin over a period of ten weeks. The serum of the rabbit producing the highest precipitin titer was used for passively sensitizing the pigs. All pigs were sensitized by serum from this rabbit at a single bleeding, with the exception of six pigs indicated by an asterisk in Table III. These pigs were sensitized by serum from a second bleeding of the same rabbit three weeks later and after four more intravenous injections of 10 mg. lactalbumin. At both bleedings the antigen was precipitated in a 1:80 dilution of serum. Sensitizing injections were uniformly 0.8 c.c. serum administered intraperitoneally.

The guinea pigs were uniform albino pigs from a single breeder and weighed from 195 to 305 Gm. They were shocked by the intravenous administration of the antigen, the ear vein being used. The volume of the dose varied from 0.33 c.c. to 1.0 c.c. Sterile precautions were used throughout. All pigs dying as a result of the injections were autopsied.

RESULTS

In Table III are shown the results of guinea pigs passively sensitized to lactalbumin and shocked by the intravenous injection of varying doses of lactalbumin. One-tenth milligram lactalbumin uniformly killed all eight pigs injected with this amount in from two to two and one-half minutes. Of six pigs treated with 0.075 mg., four died in typical anaphylactic shock and two recovered. With 0.05 and 0.03 mg., the shock was less consistently fatal. One-tenth milligram lactalbumin may then be considered the minimal, uniformly lethal dose.

Such large doses of casein were required to produce shock in pigs passively sensitized to lactalbumin that it was considered advisable to control these doses in normal pigs. The strongest extract was used, containing 55 mg. protein per cubic centimeter. This was opalescent. Furthermore, it contained some phenol which produced generalized tremors in normal pigs within fifteen seconds following intravenous injection. These tremors entirely subsided in from forty-five to sixty seconds and did not interfere with the subsequent shock. Table IV shows the results of the intravenous inoculation of this strong casein extract on normal pigs. It will be noted that one of three pigs treated with 55 mg. died of anaphylactoid shock. Eight pigs injected with

TABLE III

TITRATION OF MINIMAL UNIFORMLY LETHAL DOSE OF LACTALBUMIN IN GUINEA PIGS PASSIVELY SENSITIZED WITH 0.8 C.C. ANTILACTALBUMIN RABBIT SERUM 24 HOURS EARLIER

PIG NO.	SEX	WEIGHT (GM.)	DOSE (MG.)	RESULT DEGREE OF SHOCK
40	F	250	0.15	Died 2 min.
47	M	250	0.1	Died 2 min.
62	F	230	0.1	Died 2 min.
81	F	200	0.1	Died 2 min.
86	M	195	0.1	Died 2 min.
88*	M	220	0.1	Died 2 min.
90*	M	205	0.1	Died 2 min.
111*	M	250	0.1	Died 2½ min.
112*	M	255	0.1	Died 2 min.
42	M	275	0.075	Died 2 min.
46	M	315	0.075	++
52	F	235	0.075	Died 2½ min.
63	F	295	0.075	Died 2 min.
72	M	260	0.075	+++
89*	M	215	0.075	Died 2½ min.
41	M	255	0.05	++++
43	M	255	0.05	++++
66	M	270	0.05	Died 2 min.
68	M	245	0.05	Died 90 min.
50	F	255	0.03	++++
87*	M	180	0.03	+

*Sensitized with serum from second bleeding of same rabbit.

TABLE IV

EFFECT OF LARGE INTRAVENOUS DOSES OF CASEIN ON NORMAL GUINEA PIGS

PIG NO.	SEX	WEIGHT (GM.)	DOSE (MG.)	RESULT DEGREE OF SHOCK
61	F	275	55.0	Died 6½ min.
64	M	280	55.0	++
65	M	275	55.0	+++
92	M	185	40.0	++++
101	M	240	40.0	+
102	M	260	40.0	+++
103	M	320	40.0	++
104	M	295	40.0	+++
107	F	240	40.0	+
110	F	225	40.0	++
113	F	330	40.0	+

40 mg. showed varying degrees of response, none dying. This shock was slower in developing than true anaphylactic shock. Symptoms often did not occur until two or three minutes had passed. Dyspnea and cough were absent. The principal features were weakness and temporary paresis of the hind legs.

In Table V are shown the results in guinea pigs passively sensitized to lactalbumin and shocked by the intravenous administration of varying quantities of the strong casein extract. One of two pigs given 55 mg. died in typical acute anaphylactic shock. Of eight pigs treated with 40 mg. casein, three died in acute anaphylactic shock and showed

the typical distended lungs and beating heart at necropsy. Two others died slowly, probably from anaphylaxis, but possibly the shock was enhanced by the anaphylactoid reaction. Three showed minor degrees of shock and recovered. Doses of 27.5 and 11 mg. casein failed to produce fatal reactions. It must be concluded that, even in the presence of an anaphylactoid or foreign protein reaction, the minimal uniformly fatal dose of casein in pigs passively sensitized to lactalbumin is greater than 40 mg.

TABLE V

TITRATION OF MINIMAL UNIFORMLY LETHAL DOSE OF CASEIN IN GUINEA PIGS PASSIVELY SENSITIZED WITH 0.8 C.C. ANTILACTALBUMIN RABBIT SERUM 24 HOURS EARLIER

PIG NO.	SEX	WEIGHT (GM.)	DOSE (MG.)	RESULT DEGREE OF SHOCK
48	M	290	55.0	++++
51	F	280	55.0	Died 2 min.
49	M	255	40.0	Died 5 min.
67	M	255	40.0	++
69	M	250	40.0	Died 60 min.
70	M	240	40.0	++
71	M	220	40.0	Died 5 min.
82	F	250	40.0	Died 2 min.
84	M	210	40.0	Died overnight
85	M	225	40.0	++
45	M	250	27.5	+++
83	M	245	27.5	+
44	M	305	11.0	+±

From these results it is concluded that, since the minimal invariably fatal dose of casein in guinea pigs uniformly passively sensitized to lactalbumin is greater than 40 mg., whereas 0.1 mg. lactalbumin kills all pigs sensitized in the same manner, the casein extract must contain less than one part of lactalbumin in 400 of total protein.

PART 4

The purified casein preparation used for skin testing the infants was a 1:2,000 dilution. Therefore, any lactalbumin in it was also diluted 1:2,000. Inasmuch as the original casein was shown in the animal experiments to contain less than one part by weight of lactalbumin to 400 parts of casein, the diluted testing preparation contained less than 1:800,000. The next step was to determine whether a 1:800,000 lactalbumin solution would give positive skin tests in infants who did give positive tests to a stronger solution.

Sixteen infants who gave positive intracutaneous tests to a 1:1,000 lactalbumin solution were tested with a 1:600,000. Fifteen were entirely negative; there was one very questionable reaction. It follows that the original casein reactions were, in reality, due to casein, and could not have been caused by the less than 1:800,000 contamination that the testing solution of purified casein contained.

We wish especially to emphasize that these investigations were with infantile eczema only, and that conditions in other forms of milk sensitivity may be entirely different.

CONCLUSIONS

1. Hypersensitivity to casein is not uncommon in infantile eczema.

2. Milk in relation to allergy should be looked upon, not as one food, but as two.

3. The plan of diet to be followed in dealing with individuals hypersensitive to milk depends, to a considerable degree, upon the presence or absence of casein sensitivity.

We wish to thank Dr. Arthur Coca of New York for his helpful advice and cooperation.

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DISCUSSION

DR. ARTHUR F. COCA, Pearl River, N. Y.—I understand my function is merely to describe the method of preparation of these two fluids. All I have to say is how these solutions were made. As Dr. Hill remarked, the casein was made according to the method of Van Slyke and Baker, which is simply a modification of the original acid precipitation of the casein, washing it out and then redissolving it in sodium hydrate. The acid was added in a fine stream of tenth normal solution under constant stirring. The precipitate was treated on a Buchner funnel with alcohol ether and ether, and then kept under suction until it was dry. The casein solution that was sent to Dr. Hill was made by dissolving 10 Gm. of this powder in 75 c.c. of tenth normal sodium hydrate. To this solution, 75 c.c. of buffered saline were added, and the whole was dialyzed against 3 changes of buffered saline for 3 days and finally passed through a Seitz filter. One cubic centimeter contained 8.9 mg. nitrogen.

The lactalbumin was prepared by removing the casein with rennin and then simply dialyzing and standardizing by nitrogen content; the solution was then preserved with phenol, as usual, about 0.5 per cent, and that was the solution that was used.

DR. BRET RATNER, New York.—This problem is one that is of great interest to pediatricians and probably has greater significance from the standpoint of immunochemistry.

Dr. Pratt was kind enough to send me the paper, which I read very carefully. I wrote to him that I was very unhappy that I disagreed with some of his findings. I wonder if I can go over the slide.

(Slide) In this type of work we used about 10 or 15 times the number of animals used in this experiment. We did that for the simple reason that, when we got 10 negatives, we promptly used 10 more pigs and, to our surprise, found that, if we went on testing the pigs, we could get cross sensitizations. Furthermore, Dr. Pratt has stressed the factor of quantitative relationship in his final conclusions. I was a little surprised to see the small amount of lactalbumin he used. We used from 30 to 40 times that amount. I agree with him that the lactalbumin can be made purer than the casein. I personally would like to have seen him use more experiments and larger amounts of antigen.

This is the crucial slide (Table II). I read it over a number of times, and went over this paper with several of my colleagues. Here are animals that have been sensitized to casein using 40 times less than the amount of casein we used. We had an extraordinarily pure casein for we washed it several times more than the Van Slyke and Baker method suggests, and despite the fact that we used 30 or 40 times more casein than Hill and Pratt, we did get a few crossed lactalbumin reactions.

This slide shows the most striking example of almost 100 per cent crossed reactions, even with the small amounts of casein used for sensitization. I cannot understand how Hill and Pratt got a larger reaction with the albumin than they did with the original casein-sensitizing substance. That is something that I have never seen in animals. If there is so much albumin in that casein that the reaction to the cross substances gives a larger reaction than the original substance, then apparently there is a tremendous amount of albumin in this casein, and therefore it is not as pure as it might be.

This whole controversy depends on this crucial point: Have we caseins that are free of albumin? I daresay that in these experiments the albumin was present in large quantity in the casein used.

In Table V, these are presumed to be titrations, and you notice 2 or 3 animals used for each one. You cannot do titrations on 2 or 3 animals; you have to use 20 or 30 with each, and if you get positive reactions with the huge amount of casein used in this phase and if anaphylactoid reactions were obtained, how can Hill and Pratt determine the anaphylaxis differentiation? I would like to say in closing, therefore, that the 46 per cent casein-albumin cases that Dr. Hill and Dr. Pratt found are unquestionably in persons who are exquisitely sensitive to albumin and not to casein. The problem remains just where we left it a number of years ago, namely, that most so-called casein reactions are due to the contaminating lactalbumin in the casein and not to the casein itself. Probably 6 per cent of the cases do react to casein alone—nobody will deny that—but it seems to me that the calculations of a dilution of 1:800,000 based on the type of titration used here would justify my desire to see the work carried on further with purer antigens and more extensive titrations.

We cannot accept the conclusions drawn here today. Dr. Hill and Dr. Pratt realize, as I do, that the problem is not simple. I do hope they will continue and will report future studies. Immunochemistry is a hard taskmaster, and I am sorry I have had to criticize this study.

DR. HILL.—I expect it is a very good thing at a medical meeting to have some disagreement, because if there were none, there would be no fun; we would all be bored by it. However, I am nonetheless disappointed that Dr. Ratner does not like this animal work, because it was really put on especially for him. I have been trying for several years to convince him that casein sensitivity is not very uncommon in infantile eczema. I am going to let Dr. Pratt answer his objections to the animal work, because he is the one who did it.

Dr. Ratner said that these patients who gave positive tests to casein were probably exquisitely sensitive to lactalbumin. There was no patient in the group who was exquisitely sensitive to lactalbumin. Very few of these babies with eczema are ever exquisitely sensitive to lactalbumin. It is a mild sensitivity. They do not give large skin tests. They are all drinking a quart of milk a day and thrive on it, except for eczema, and I doubt very much if you could shock them by injecting milk. They are going through a temporary phase of abnormal immunology, and while they are going through it, they are mildly sensitive to milk and they have eczema.

Then there are two other points. In the first place, it would certainly be peculiar in casein-testing 70 babies who were sensitive to lactalbumin to get so many negative casein reactions if the casein reactions were due to traces of lactalbumin in the casein. Second, goat's milk and cow's milk casein will always react exactly the same and every time there will be a positive reaction to goat casein when there is one to cow casein, but there is only a little more than half crossed reactions between goat and cow lactalbumin. Therefore, if you use goat casein for testing, many times it does not make any difference whether the goat casein is contaminated with lactalbumin; the casein reactions are just as authentic.

Dr. Ratner said a number of years ago that he doubted if it was possible to get casein absolutely pure. He was the first one to bring this out, and it was a very valuable thought because I do not think anybody had ever realized it before.

The casein that we used, as we showed, did have traces of lactalbumin in it. We think we have shown, however, that the amount present was too small to have given positive skin tests in this particular group of patients, and that the tests we obtained were in reality due to casein.

In young babies, milk is a very important allergen. Therefore, I think it is of some value to be able to tell whether or not casein sensitivity is present in any given case, because this does influence treatment to a certain extent, as I pointed out in the beginning.

DR. PRATT.—I want to make 3 points very briefly for Dr. Ratner. First, as to the number of pigs. If 8 or 10 pigs in a row do identically the same thing without any failures, it is fairly satisfactory evidence.

Second, as to that crucial experiment of which you spoke, where the pigs actively sensitized to casein reacted more strongly to the lactalbumin solutions than to the casein, that was perfectly true. Lactal-

bumin is a much more powerful antigen than casein. The minute quantities of contaminating lactalbumin in the casein extract were sufficient to sensitize the pigs to lactalbumin. However, having reacted to lactalbumin, the pigs could still be killed with casein, as happened in two instances. In other words, they were specifically sensitive to 2 antigens.

Third, it is true that the casein extract was so strong it produced anaphylactoid reactions in normal pigs; but these reactions were very slow in developing. They occurred 3, 4, and 5 minutes after the intravenous injection. Even a specific anaphylactic reaction followed by a nonspecific anaphylactoid reaction failed to kill these pigs.

I think we were very conservative when we said the contamination was only 1 in 400; it was probably very much less than that.

ALLERGY TO PANCREATIC TISSUE EXTRACT, WITH REPORT OF TWO CASES

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DURING the past few years an increasing number of reports have appeared on instances of acquired allergy to biologic products such as insulin, pituitary extract, liver extract, thyroid extract, and epinephrine. All of these reports were on persons who had received the biologic product for therapeutic purposes over a variable period of time before suddenly developing a reaction. The reaction most frequently consisted of a skin eruption, usually generalized urticaria and angioneurotic edema, followed by occasional fever and dyspnea of varying degrees of intensity. At times nasal, ophthalmic, and asthmatic symptoms also occurred. It is agreed that these reactions constitute, for the most part, an acquired or induced form of allergy in many ways analogous to serum reaction. The following is a report of acquired allergy to pancreatic tissue extract* observed during the course of treatment in two patients with peripheral vascular disease. This report is believed to be the first report of acquired allergy to tissue extract.

CASE REPORTS

CASE 1.—F. C. R., Italian, male, aged 42 years, a heavy smoker, was admitted to the hospital in March, 1939, complaining of pain, redness, and swelling of the left big toe. He gave no history of asthma, hay fever, urticaria, eczema, or of allergy to drugs. There was no family history of allergy. The general physical examination was negative. The big toe was swollen, red, and tender midway up to the first metatarsal. Both feet were reddish and cold. The skin was glossy and moderately tense; the hair, sparse and brittle; the nails, thick and yellow. The dorsalis pedis and posterior tibial vessels were not palpable. On elevation the feet became pale. The Samuel test was positive bilaterally. Oscillometric readings were equal and diminished on both sides. A roentgenogram of the feet showed no evidence of bone destruction. The proximal phalanx of the left great toe showed some absorption of lime salts. No calcified formation in the blood vessels of the feet was seen. Urine, blood count, serology, and blood chemistry tests were negative. No blood eosinophilia was present.

On the basis of the above findings the diagnosis of thromboangiitis obliterans of both legs was made, and suitable treatment was instituted. The patient received 4 c.c. of deinsulinized pancreatic tissue extract twice a week from April 24,

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*The extract referred to is Sharpe & Dohme pancreatic tissue extract, No. 568, which is described as a purified acid-alcohol extract of beef pancreas free of insulin. It is used for its depressor effect on arterial blood pressure.

1939, to June 9, 1939, and three times a week from June 9 to July 6, a total of twenty-six injections. Treatment was interrupted for one month and on August 12 it was resumed and continued until Dec. 26, 1939; the patient received 4 c.c. of tissue extract twice a week, or a total of thirty-eight injections. Altogether he had been given intramuscular injections of tissue extract for an approximate period of eight months, a total of sixty-four injections without any untoward reaction. However, within a few minutes following the usual injection on December 26, he developed a very severe and sudden reaction. The face became suffused and red; the entire body became covered with urticarial wheals; the legs and eyes became puffy and swollen. The patient complained of intense itching of the skin, eyes, and nose; his breathing became difficult and typically asthmatic, the wheezing being audible without a stethoscope. His condition seemed serious and necessitated the immediate administration of several doses of epinephrine hydrochloride 1:1,000 subcutaneously. His condition improved, with the exception of the angioneurotic edema of the hands and fingers which persisted for twenty-four hours. At the time of this reaction the patient showed an eosinophilia of 7 per cent.

In view of the seriousness of the reaction and in order to study the possible mechanism which produced it, treatments were discontinued for about one month. On Feb. 5, 1940, however, he was given another injection of 3 c.c. of tissue extract, without developing any reaction. Five days later, upon receiving the next dose of tissue extract, the patient developed another general reaction, similar to, but less severe than, the first. Treatments were resumed on February 20 and continued in doses of 3 to 4 c.c. at weekly intervals for three weeks, then every second day until March 20 (a total of nine injections) without any untoward symptoms. On April 23 the patient was given an injection of 4 c.c. of tissue extract, and the treatments were continued every five days without any reaction.

CASE 2.—W. H. G., male, aged 41 years, was admitted to the hospital in November, 1939, complaining of cramplike pains in the calves of both legs, especially noticeable on walking. This condition had been present for several years, becoming progressively worse, so that at the time of admission the limit of his walking tolerance was about one-half of a block. His feet were very cold in the winter. Within the past year, he had experienced numbness of the fingers of the right hand and pain under the nails. When immersed in cold water, the fingers became very painful. He smoked to excess. There was no history of asthma, hay fever, eczema, or allergy to drugs. There was no family history of allergy.

The general physical examination was negative, and examination of the upper extremities was negative. There were no demonstrable trophic changes in the nails of the toes. On dependence the toes assumed a reddish hue which quickly faded on elevation, leaving a cadaveric pallor. Samuel's test was negative. Dorsalis pedis, posterior tibial, and popliteal vessels were not palpable. Femoral pulsations were apparently normal. Oscillometric readings were zero in both the right and left foot and ankle and were reduced in both calves.

General treatment for thromboangiitis obliterans of both legs was instituted. The patient received 3 c.c. of deinsulinized pancreatic tissue extract intramuscularly on Nov. 11, Nov. 13, and Dec. 21, 1939. These injections were discontinued for about one month and resumed on Feb. 5, 1940, without any reaction. On February 10, after receiving 3 c.c. of tissue extract, the patient developed an immediate reaction consisting chiefly of generalized urticaria which responded to the subcutaneous administration of epinephrine hydrochloride 1:1,000. At that time he showed 2 per cent blood eosinophilia. The next dose was given on February 21 and twice a week until March 20, without any reaction. The injections were resumed on April 23, 1940, without any untoward reactions.

IMMUNOLOGIC STUDIES

In an attempt to study the possible mechanism of these allergic reactions to tissue extract, the following examinations were carried out.

1. Determination of Skin Sensitivity to Tissue Extract (Table I).—Intradermal skin tests were performed with various dilutions of tissue extract on both patients. The first patient (F. C. R.) had two severe allergic reactions, the first on Dec. 26, 1939, and the second on Feb. 10, 1940. The second patient had only one allergic reaction, on Feb. 10, 1940. The tests were carried out at various intervals following these reactions in order to determine the presence and nature of any changes. Table I indicates that both patients showed definite evidence of skin reactivity to various dilutions of tissue extract shortly after they developed the allergic reaction. Furthermore, they continued to show a positive skin reaction to tissue extract up to the last time they were tested (April 23, 1940). While it is very likely that the marked positive reactions to concentrated tissue extract are significant, these must be disregarded in view of the fact that the control reacted slightly to this concentration. It is interesting to note that neither of the two patients gave an ophthalmic reaction to diluted tissue extract. Six normal controls gave negative skin reactions to 1:10 dilutions of tissue extract.

TABLE I

TESTS ON PATIENTS AND CONTROLS TO DETERMINE SKIN REACTIVITY TO TISSUE EXTRACT

CASE	PATIENT	DATE	TISSUE EXTRACT					GLUE INTRADERMAL	SALINE INTRADERMAL
			CONCENTRATED	1:10		1:100			
			INTRADERMAL	INTRADERMAL	EYE	INTRADERMAL	EYE		
1	F. C. R.	Jan. 17, 1940	+++	++	0	+	0	0	0
		Feb. 12	++++	+++	+	++	0	0	0
		Feb. 23	+++	++	0	+	0	0	0
		April 23	0	+++	0	++	0	0	0
2	W. H. G.	Feb. 12	+++	+++	0	++	0	0	0
		Feb. 23	+++	+++	0	+	0	0	0
		April 23	++	++	0	++	0	0	0
	3 normal controls	Feb. 12	+	0	0	0	0	0	0
	6 patients treated with tissue extract	Feb. 25	++	0	0	0	0	0	0

2. Passive Transfer (Table II).—The presence of skin-sensitizing antibodies or reagins in both patients is demonstrable by passive transfer. Reagins were found in varying amounts at the time the allergic reaction was manifested and continued to be present for a period of several months thereafter. It is interesting to note that these reagins are

TABLE II

PASSIVE TRANSFER TESTS TO DETERMINE PRESENCE OF REAGINS IN TWO PATIENTS SHOWING ALLERGIC REACTION TO TISSUE EXTRACT

NO.	SUBSTITUTE	DATE	CASE 1 (F. C. R.)					CASE 2 (W. H. G.)				
			SERUM	TISSUE EXTRACT 1:10	SERUM + TISSUE EXTRACT 1:10	SERUM + TISSUE EXTRACT 1:100	SERUM + GLUE EXTRACT	SERUM	TISSUE EXTRACT 1:10	SERUM + TISSUE EXTRACT 1:10	SERUM + TISSUE EXTRACT 1:100	SERUM + GLUE EXTRACT
1	W. C.	Jan. 17, 1940	0	0	++	++	0					
		Jan. 19	0	0	++	+	0					0
		Feb. 6	0	0	++	+	0					0
		Feb. 10	0	0	+++	++	0	0	0	++	±	
2	A. C.	Jan. 17	0	0	+++	+	0					
		Jan. 19	0	0	+	+	0					
		Feb. 6	0	0	+	+	0					0
		Feb. 10	0	0	+++	+	0	0	0	+++	++	
3	L. F.	Jan. 17	0	0	++	++	0					
		Jan. 19	0	0	++	+	0					
		Feb. 6	0	0	+	+	0					0
		Feb. 10	0	0	++	++	0	0	0	++	+	
4	C. L. A.	Jan. 17	0	0	+	+	0					
		Jan. 19	0	0	+	+	0					
		Feb. 6	0	0	++	++	0					0
		Feb. 10	0	0	+++	++	0	0	0	++	++	
5	E. D.	April 23	0	0	+++	++	0	0	0	++	++	0

demonstrable at periods when both patients failed to manifest allergic reactions despite the continued administration of tissue extract twice a week.

The passive transfer tests were conducted in accordance with the usual technique. A small amount of the serum of each patient was transferred to several areas of the skin of four normal substitutes. The sensitized sites were tested intradermally after a lapse of from twenty-four to forty-eight hours with tissue extract in dilutions of 1:10 and 1:100. One site in each determination was tested with glue extract as a control and found to be negative.

3. Organ or Species Source Sensitivity (Tables III and IV).—The pancreatic tissue extract employed was obtained from beef. The following study was made in an attempt to determine whether sensitivity to tissue extract in the instances reported was due to pancreas protein, i.e., an organ sensitivity, or to the muscle protein of the animal from which the pancreas was obtained, i.e., a biologic source sensitivity. Direct and passive transfer tests were carried out, employing extracts of various kinds of pancreas and muscle protein, such as beef, pork, and chicken.

TABLE III

INTRADERMAL TESTS TO DETERMINE ORGAN OR SPECIES SOURCE SENSITIVITY OF SKIN TO TISSUE EXTRACT

CASE	NAME	DATE	MUSCLE PROTEIN			PANCREAS			CONTROL
			BEEF	PORK	CHICKEN	BEEF	PORK	CHICKEN	KAPOK
1	F.C.R.	Jan. 17, 1940	+++	0	0	+++	++	0	0
		Feb. 10, 1940	+	0	0	+	++	0	0
2	W.H.G.	Feb. 10, 1940	0	0	0	+++	++	0	0
	Control		0	0	0	0	0	0	0

TABLE IV

PASSIVE TRANSFER TESTS ON SIX SUBSTITUTES TO DETERMINE PRESENCE OF REAGINS

SUBSTITUTE	CASE	DATE (FEBRUARY, 1940)	MUSCLE PROTEIN			PANCREAS			CONTROL
			BEEF	PORK	CHICKEN	BEEF	PORK	CHICKEN	KAPOK
1	F.C.R.	6	0	0	0	+++	+	0	0
	F.C.R.	10	0	0	0	++	+	0	0
	W.H.G.	10	0	0	0	+++	+	0	0
2	F.C.R.	6	0	0	0	++	++	0	0
	F.C.R.	10	0	0	0	++	+	0	0
	W.H.G.	10	0	0	0	++	+	0	0
3	F.C.R.	6	0	0	0	++	±	0	0
	F.C.R.	10	0	0	0	+	±	0	0
	W.H.G.	10	0	0	0	+++	++	0	0
4	F.C.R.	6	0	0	0	++	+	0	0
	F.C.R.	10	0	0	0	++	++	0	0
	W.H.G.	10	0	0	0	+++	++	0	0
5	F.C.R.	6	0	0	0	++	++	0	0
	F.C.R.	10	0	0	0	+	+	0	0
	W.H.G.	10	0	0	0	+++	±	0	0
6	Normal	10	0	0	0	0	0	0	0

The results are given in Tables III and IV. On Jan. 17, 1940, or two weeks following the initial reaction, and also a few hours following the second reaction (February 10), the first patient (F. C. R.) gave positive direct skin reactions to beef muscle, beef pancreas, and pork pancreas. The other skin tests were negative. Passive transfer tests (Table IV) employing his serum indicated no reagins against beef muscle protein or chicken pancreas but definite reagins against beef and pork pancreas. Repetition of the tests on this patient and with his serum a few hours following his second allergic reaction to the injection of tissue extract yielded similar results. The tests on the second patient (W. H. G.) a few hours following his allergic reaction indicated both skin sensitivity and the presence of skin-sensitizing antibodies or reagins to beef and hog pancreas protein. It is interesting to note that negative passive transfer tests were obtained to chicken pancreas. Most of these findings would seem to point to the possibility that in these instances, acquired sensitivity to pancreatic tissue extract is an organ sensitivity and not a biologic source sensitivity.

4. Presence of Associated Antibodies.—In some forms of acquired allergy, such as induced allergy to horse serum, worms, insulin, posterior pituitary solution, and liver extract, there develop in the serum of the patient other antibodies such as precipitins and anaphylactic antibodies. Precipitin tests were performed using the serum obtained from each of the two patients shortly after the allergic reaction. This serum was introduced into test tubes in various concentrations and overlaid with a small quantity of tissue extract solution. Precipitins against tissue extract could not be demonstrated.

Several guinea pigs were injected with the serum of each patient in an attempt to discover whether any anaphylactic antibodies were present. In no instance could anaphylactic shock be produced later by the intravenous injection of tissue extract into these animals. It would seem, therefore, that neither of these patients had developed anaphylactic antibodies against tissue extract.

5. Experimental Induction of Allergy to Tissue Extract.—Studies similar to those indicated above were carried out on a total of seven patients suffering with peripheral vascular disease. These patients had been receiving 3 c.c. of tissue extract once or twice a week over a period of from four to twenty-four months. One patient was definitely atopic as indicated by the presence of a family history of allergy and a personal history of asthma. None of these seven patients developed any kind of allergic reaction following injections with tissue extract. Direct skin tests showed a slight positive reaction to concentrated tissue extract but no reaction to a 1:10 and a 1:100 dilution of this extract. Passive transfer was uniformly negative in all instances. It would seem, therefore, that neither a clinical sensitivity nor reagins to this tissue extract can be produced at will in the human being.

6. Further Studies on a Previously Reported Case of Allergy to Liver Extract.—In order to throw additional light on the possible mechanism of acquired allergy to biologic products, further studies were carried out on a patient whose acquired allergy to liver extract was reported by the writer[1] in 1938. This patient had been taking weekly injections of liver extract in 1935 for a year without any untoward reactions. The treatments were discontinued for three months. Following the third injection after resumption of treatment he developed a severe allergic reaction characterized by asthma and urticaria. However, a few months later (October, 1936) it was found he could tolerate again injections of the same liver extract. He has continued to take these injections for the past four years practically without interruption and without showing any allergic manifestations.

Direct skin tests and passive transfer in March, 1940, or nearly five years following his original allergic reaction to liver extract, showed negative skin tests and negative passive transfer for reagins against liver extract.

DISCUSSION

This discussion will be limited to the following points: (1) the nature of the observed reactions; (2) the time of their occurrence; (3) the experimental production of allergy to biologic products; (4) the role of reagins in these reactions; (5) organ or biologic source sensitivity; and (6) the practical application of the principle of desensitization in these instances.

It is obvious from what precedes that the type of reaction developed by these two patients is a form of acquired or induced allergy to tissue extract. This reaction bears the characteristics of acquired serum reaction. Both patients took the injections of tissue extract at first without showing any untoward reaction. In other words, previous exposure or contact was necessary to produce the allergy. The reaction in one patient (F. C. R.) was, in our opinion, definitely of the atopic type, namely, asthma and urticaria. This patient gave a previous history of attacks of coughing, and, while the hereditary factor is not clearly evident, we have reasons to consider him atopic. The reaction in the second patient was clearly more of the clinical reaction observed in acquired serum allergy.

A study of many of the reported instances of allergy to biologic products reveals a very interesting observation. Many of the patients had been receiving insulin,[2] liver,[1] pituitary,[3] and (in the cases reported in this paper) tissue extract over a fairly long period of time at regular intervals without any allergic manifestations. The allergic reaction usually developed after the second or third injection following a

lapse of time during which the patient did not receive any treatment. This observation is of some interest because it reminds one of the presence of an incubation period in anaphylaxis preceding the effective shock dose.

Attempts at experimental production of allergy to biologic products by repeated administration over a period of time have been no more successful than similar attempts at inducing at will many other forms of allergies in the human being. Obviously, only a small percentage of the large number of patients receiving biologic products such as insulin, tissue extract, etc., develops an allergy to them. Indeed, these instances are so rare that, when they occur, it is considered worth while to report them. It seems, therefore, that exposure alone is not sufficient to bring about the development of an acquired sensitivity of this type. Nor is the presence of an hereditary or atopic shock tissue, such as is found in atopic persons, a factor, for we have been just as unsuccessful in sensitizing atopic persons as others to biologic products.

What is the role of specific skin-sensitizing antibodies or reagins in acquired allergy to biologic products? Both of our patients showed definite specific skin-sensitizing antibodies or reagins at the time of their reaction. Both patients, however, continued to show these reagins in the same concentration for months following their reaction and at periods when they received by injection numerous doses of tissue extract without any untoward manifestations. Apparently the quantity of reagins present had little to do with the severity of the allergic reaction, for the patient (F. C. R.) who manifested the severest reaction showed by passive transfer tests a comparatively smaller quantity of tissue extract reagins than the second patient (W. H. G.) who had a milder general reaction. These reagins are incidental immune bodies in acquired allergy and play no role in the acquired allergic reaction. They differ from atopic reagins in that they are less permanent and are found in smaller concentration.

The evidence points to the presence in these instances of a sensitivity to pancreatic tissue protein and not to the muscle protein of the animal from which the pancreas was obtained. In other words, we are dealing with an organ and not a biologic source sensitivity. This evidence is based largely on two considerations: first, the presence of reagins in the serum of our two patients sensitive to the proteins of the pancreas regardless of whether it was hog or beef pancreas and the absence of reagins to the muscle protein of these animals; and, second, on clinical grounds, the demonstration that such patients will continue to show clinical sensitivity when given pancreatic tissue extract regardless of its source, i.e., the mere changing of the batch of the extract will usually not render its administration harmless. One should point out the

possibility that tissue extract or pancreatic extract may contain a very small amount of the serum of the animal from which it is obtained and that the small quantity of serum may play a role in the immunologic studies carried out in this investigation. May it be that the reason some insulin-sensitive patients are found to tolerate occasionally insulin of a different source is because the patient frequently loses his insulin sensitivity spontaneously? There is no prediction when an allergic reaction to a biologic product may develop or when it will fail to develop. A patient may show a reaction to an injection today, none for the next two or three injections, and then another reaction later. These patients do not react with the same unfailing constancy following exposure to the respective allergens that atopic persons, such as hay fever patients, show when exposed to pollen.

Similarly, spontaneous loss of sensitivity may explain the reports of successful, complete desensitization which occasionally appear in the literature. We tried this method of desensitization in a previously reported instance of liver extract allergy and failed. If we had attempted so-called desensitization in our first patient (F. C. R.) by repeated administration of small doses of tissue extract shortly after he developed the allergic reaction, we naturally would have interpreted his subsequent tolerance of tissue extract as being caused by this procedure and not by a spontaneous loss of sensitivity as the facts indicate. However, it may be possible that extensive treatment with a given extract may exhaust the majority of circulating antibodies or in some other manner lead to a state of hyposensitization. It must be pointed out that there is no evidence that there was any diminution in the amount of demonstrable reagins in our patients as a result of continuous treatment with tissue extract. As a matter of fact, there is evidence that there was an actual increase in these reagins at first, a finding analogous to that obtained in the treatment of hay fever.

SUMMARY AND CONCLUSIONS

1. Two instances of acquired allergy to tissue extract are reported.

2. These reactions simulate acquired serum reaction.

3. The mechanism of induced allergy to biologic products is not clear.

4. The allergic reaction usually occurs after the second or subsequent treatment following a period of rest which was preceded by a long period of treatments.

5. This reaction cannot be produced at will.

6. Various immune bodies such as reagins, precipitins, anaphylactic antibodies, etc., which may be demonstrated to be present in these instances are incidental antibodies and play no role in the reaction.

7. It is probable that an organ sensitivity and not a species source sensitivity is present.

8. Desensitization is not possible in these instances. Spontaneous, unexplainable loss of sensitivity to biologic products occurs frequently.

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MAY BUILDING

DISCUSSION

DR. LOUIS TUFT, Philadelphia.—The two cases reported by Dr. Criep indicate additions to an increasing list of foreign agents that are capable of inducing sensitivity after repeated injection. As he has pointed out, they are essentially like the artificially induced serum allergy and resemble somewhat anaphylaxis in the guinea pig. This report emphasizes the fact that when any foreign serum, drug, or extract is injected into a large group of persons, some of these persons may develop a sensitivity to the injected agent so that reinjection causes a reaction, usually of the urticarial type.

As Dr. Criep pointed out, the important determining factor is the lapse between periods of injection, since this provides an incubation period during which the person can become sensitized, just as happens in the experimental guinea pig. As long as the injections are given at short intervals, nothing will happen. But if one stops them for a longer period and then starts again, this provides a period sufficient for sensitization to occur, and this will be evident upon reinjection.

The second interesting point relates to the matter of so-called desensitization in these cases. It is well known that the patients who develop serum sickness will recover from it without its leaving any trace of its presence except possibly for a positive skin test. Such patients can be reinjected, as Park has shown, without any immediate reaction. Hence, as in Dr. Criep's case, it is possible for the patient to recover from a reaction and later to take injections of the same material without any reaction whatsoever. If desensitization had been instituted, it would have been given credit for the patient's ability to take subsequent injections when, as a matter of fact, the patient could have taken them anyhow. It is important, therefore, in evaluating such attempts at desensitization to be sure that the patient continues to get reactions after each subsequent injection and that the induced sensitivity is of a more permanent, rather than temporary, character.

DR. SAMUEL M. FEINBERG, Chicago.—We have had experience with four or five patients with allergy to liver injections which resembles in most respects the experience of Dr. Criep. I think all of these patients were atopic; their reactions occurred after repeated injections, not after first; and most of them desensitized themselves spontaneously. We believe that small increasing doses were instrumental in causing desensitization in one patient in whom we had caused repeated anaphylactic attacks.

Our experiences with these types of allergy differ somewhat from those of Dr. Criep. We have reason to believe that we have, in addition to the organ type of allergy, species specificity and specificity actually, at least in one patient, to the antianemic fraction.

Dr. M. Murray Peshkin, New York.—This study strongly points to sensitization as being independent of the heterogenous or homologous serum and muscle tissue proteins. It is safe to state that many allergists have seen one or two patients who reacted to liver extract. An interesting case in point is that of a female schoolteacher who could not ingest nor be injected with the smallest quantity of liver extract without having asthmatic symptoms. However, the ingestion of chicken, beef, or pork was tolerated. Another case is that of a male adult with asthma of bacterial origin who subsequently became afflicted with chronic lymphatic leucemia. Asthma almost immediately followed the third injection of a liver extract preparation. Several subsequent injections of the same or other liver extracts resulted in acute asthma. The ingestion of liver extracts, as well as the muscles of various animals, was tolerated. The skin test to liver extract was positive in the latter patient only, and reactions to the muscles of various animals were negative in both. These and other observations seem to confirm the observations and conclusions made by Dr. Criep.

Dr. Criep makes the statement that he does not know the mechanism responsible for this type of acquired hypersensitiveness. Whether or not he made reference in his paper to the nature of the mechanism in the so-called "atopic" type of hypersensitiveness is not important. The fact still remains that his statement regarding the induced type of hypersensitiveness does carry with it the inference that the latter is different and apart from the "atopic" type of hypersensitiveness and that perhaps even the mechanisms underlying each type are also different. Separating acquired from "inherited" (atopic) hypersensitiveness and, in turn, both of them from allergy merely adds confusion to a phase of the subject already replete with confusion. Allergy is the foundation for all types of sensitivity regardless of their origin. I am convinced that induced tissue allergy and other acquired sensitizations fundamentally are no different from the sensitizations commonly encountered. The continued practice of separating one type of allergic sensitization from another on a basis of whether one is an acquired or the other "inherited" merely helps to retard progress toward a logical understanding of the subject of allergy.

There can be no fault found with the introduction of the atopic concept of hypersensitiveness but rather with the stubborn persistence on the part of those who still cling to the original concept despite the many advances in research made in clinical and experimental allergy which most definitely throw the weight of evidence away from the atopic to that of the broader and more logical concepts embraced in the allergic theory. It is about time the term "allergy" be the only term employed to describe all types of altered reactivity of the cells. As a matter of record, Doerr, Schick, Zinsser, and others, in their classification of hypersensitiveness in human beings, believe that the similarities between allergy (human) and anaphylaxis (animal) are of more significance than the differences. Nevertheless, in the light of present-day advances, there are those who still insist on the rigid separation of allergy (atopy) from anaphylaxis, basing their contention on

the establishment of heredity as the sole determining factor. This extreme attitude, which discards many similarities in favor of a few differences, is illogical unless these differences can be shown to be fundamental. Zinsser and Schick do not believe that they are, and over a decade ago it seemed to them that such rigid separation was likely to impede understanding of the significance of many clinical manifestations. Zinsser succinctly summarized the pertinent facts regarding this phase of the subject when he stated: "It has become already apparent that many of the observations necessary for an understanding of these conditions cannot be made on animals. It is a peculiarity of hypersensitiveness that identical agents, by a probably identical mechanism, produce widely divergent effects in different species of animals, and many of the problems underlying the clinical manifestations of human hypersensitiveness can therefore be solved only by observation of the human being, and by conservative experiment upon clinical material, so far as this may be safely done. . . . Furthermore, the facts of hypersensitiveness, with the insurmountable similarity as to specificity, symptomatic complex, laws of desensitization, etc., teach us that such specific cellular reactiveness may be acquired against materials separated in regard to chemical structure as the simple iodides and the complex proteins, and we are forced to conclude that there is a common principle underlying all of them."

Dr. William C. Service, Colorado Springs.—I have been impressed with some of these cases of reactions to tissue extract in which the time element was involved. I have never seen any of these reactions occur where you give the patient rest periods, in which the second reaction has occurred in less than seventeen days. I do not know whether there is a definite time period which is involved in regard to this secondary sensitization, but I should like to know if Dr. Criep has ever noticed that the time element is a factor.

Dr. Armand Cohen, Louisville.—I should like to ask if Dr. Criep separated his material into the various fractions. Dr. Frank Simon and I, in our work in insulin sensitivity, were impressed by the extreme specificity in that we found certain patients sensitive, for instance, to pancreatic amylopsin and not sensitive to insulin or any other protein in the pancreatic material.

Dr. George L. Waldbott, Detroit.—A case of interest in connection with this paper is that of a woman who suffered severe allergic shock following an injection of theelin. Since then she developed the same syndromes (urticaria, sneezing, and asthma) every four weeks shortly before menstruation.

In this discussion we do not have to confine ourselves to endocrine preparations. We all have seen allergic shock from simple organic or inorganic chemicals. Several years ago I reported such a case following an intravenous injection of urea and quinine. In this case, the time interval of three weeks and more seemed to be instrumental in the development of sensitivity. I know of other such instances caused by the use of neoarsphenamine, sodium salicylate, and sodium cacodylate.

Dr. Tuft quoted Dr. Park as saying that, following serum sickness, sensitization usually disappears. I have seen several patients who de-

veloped serum sickness within the five- to ten-day intervals following the injection of horse serum; subsequent injections of horse serum induced allergic shock.

DR. BRET RATNER, New York.—I want to congratulate Dr. Peshkin for so ably emphasizing the important controversy that arises from Dr. Criep's statement that this particular reaction that he has observed is different from what we see in anaphylaxis. I think one of the great pitfalls of this whole controversy is the fact that the methodology used has not been thoroughly understood; that is, if you try to transfer passively human serum that might contain an anaphylactic antibody to one, two, or three animals, you are doomed to failure; but if you use ten or fifteen animals, you probably will find one or two that will respond. This probably is caused by some qualitative difference in the human serum.

An accelerated serum reaction is not different from an anaphylactic accelerative reaction. I do not know why anybody has ever attempted to separate serum sickness from anaphylaxis, because every single phase of the serum sickness problem has its counterpart in lower animal anaphylaxis.

DR. MARION B. SULZBERGER, New York.—I do not believe any of us here any longer entertain any doubts as to the existence of organ-specific sensitizations or to the fact that human beings and animals can become sensitized to liver, to something specific and peculiar to the liver, or to organ-specific factors of the pancreas, the brain, the pituitary, and many other organs. I believe these organ-specific sensitivities in man have been demonstrated conclusively by Dr. Louis Tuft, Dr. Frank Simon, Dr. Criep, and others.

The sole, minor criticism I have of Dr. Criep's paper deals with his terminology. I believe that it will be confusing to readers to call the specific pancreatic extract merely "tissue extract." After all, an extract which is made from the lungs, the liver, or the kidneys is also a "tissue extract." I believe it would be an improvement if Dr. Criep called his extract an "extract of the pancreas." Moreover, I believe that the term "biologic source" is also confusing when applied to "species-specific factors." After all, the material which is "organ-specific" is also derived from a biologic source. I believe, therefore, that if he would use the term "species-specific factor" or "organ-specific factor" for these two different allergenic factors, it would make it easier for readers who are not as well acquainted with the subject as he is.

It would indeed be a pity to close this discussion without calling attention to the wider, deeper, and possibly very important fundamental considerations which are implicit in this contribution. If human beings and animals can become sensitized to organ-specific factors when these are administered from without, cannot the organ-specific factors which the organs are themselves producing within the individual also, on occasion, be liberated and slightly altered and thus become antigenic and themselves produce autogenous organ-specific sensitization? Is it not possible that these sensitivities to specific substances derived from the human pancreas, the human liver, or the human kidney, all within the human being, may give rise to organ-specific antibodies which then may be able on occasion to attack the specific organ substances in vivo

and to damage the particular organs in situ? Is it not conceivable that the recognition of this possibility may prove very important for our fundamental concepts of the pathogenesis of certain chronic or chronic recurrent diseases of obscure etiology?

I think the work of Masugi, Burky, Rivers, Schwentker, and many others demonstrates that this possibility has to be considered in the genesis of diseases of the eye, the kidney, the cerebrospinal axis, and perhaps in other diseases as well. I have elsewhere discussed at some length these interesting possibilities and their possibly far-reaching implications.*

DR. HARRY S. BERNTON, Washington, D. C.—I have two questions to ask. What was the family history of the two patients who were actively sensitized? Did you try feeding experiments with the organ itself in the two patients?

DR. WARREN VAUGHAN, Richmond.—Years ago Dr. Blackley claimed that cat asthma was due to pollen brought in on the fur of the cat. When someone remarked that city cats, which were not out in the grasses, also caused cat asthma, he said, "Yes, but they have been eating mice which have been out in the fields." Now today we do not keep on criticizing Blackley for having had such an idea.

I feel that the theory of atopy and the differentiation of the allergic diseases proposed by Dr. Coca has stimulated thought for progress in allergy. It has stirred up much controversy and has clarified our understanding. Theories are but stepping stones in the progress of knowledge. As we step from one stone to a new one, we pass the last one; no theory is the last theory. I do not know how Dr. Coca feels about his classification at the present time. I do not know of any recent restatement that he has made on the subject. Argument today about this theory which was promulgated many years ago seems to me as unnecessary and as futile as would be any argument now over Blackley's theory of cat asthma.

DR. CRIEP.—I thought I limited my presentation to a demonstration of two cases of acquired sensitivity to pancreatic tissue extract and nothing else and was steering clear of controversial discussion.

It was not and it is not my intention to bring into this discussion the question of the validity of one or another form of allergic classification. I am not particularly concerned with atopy or the identity of anaphylaxis and allergy in this discussion. These are subjects about which we all hold opinions which are entitled to tolerant consideration. I did not make an issue of any of these debated topics. I respect many of the observations of my early teacher, Dr. Coca, but I likewise have high regard for many of the conclusions which my friends Dr. Peshkin and Dr. Ratner have drawn over a period of years. But in this particular paper, I wished merely to present two instances of acquired allergy to pancreatic extract and to indicate certain findings in a clinical investigation of these two patients. These results need not necessarily apply, and indeed from my own observations do not always apply, to instances of acquired allergy.

When it comes to the question of passively sensitizing animals, I am well aware that Dr. Ratner has been able to demonstrate the possibility

*Dermatologic Allergy, Springfield, Ill., 1940, Charles C Thomas.

of this procedure. We have been able to sensitize passively animals with human sera by transferring anaphylactic antibodies; but there, again, all I said was that the sera of these two patients did not have enough anaphylactic antibodies to give passive sensitization. I am sure Dr. Ratner and most of you will agree that this may happen.

In answer to Dr. Cohen's question, unfortunately we did not separate any of the various forms of pancreas proteins in order to perform experiments with these various fractions.

Dr. Waldbott is absolutely right. This type of reaction is analogous to the type of reaction which we see in induced allergy to drugs; there is no difference between the two.

Dr. Sulzberger is also right. The only reason I referred to the extract as tissue extract throughout this discussion was because I was trying to save time. In the paper, of course, we refer to this extract as deinsulinized pancreatic tissue extract, Sharp & Dohme, No. 568. Dr. Sulzberger's point about referring to this as a species-specific or an organ-specific sensitivity is, I think, very well taken. My terminology was an adaptation of that used by some of my colleagues, Dr. Simon, Dr. Tuft, and others, who have been using in the literature the terms "organ" and "biologic source" sensitivity. I shall gladly accept Dr. Sulzberger's nomenclature because it is clearer.

In reply to Dr. Bernton's questions, it must be stated that the first patient gave us reasons to believe he was atopic; the second was a perfectly normal person.

SKIN REACTIONS

X. Preseasonal Treatment of Hay Fever by Electrophoresis of Ragweed Pollen Extracts Into the Skin: Preliminary Report

Harold A. Abramson, M.D.
New York, N. Y.

INTRODUCTION

IT HAS been claimed repeatedly that intradermal vaccination is a preferred route for active immunization.[1] This idea has been adopted by certain investigators in the field of therapeutics of hay fever. It is advocated that the intradermal route be used not only for coseasonal therapy, but also for preseasonal treatment. Since the method of electrophoresis transports materials directly into the dermis through the pores of the skin,[2] it is an ideal method of administering substances intradermally without injury. This fact is shown clearly by the whealing reaction following histamine and by the blanching reaction following epinephrine, if either is administered by electrophoresis for short periods under proper conditions.

Not only may molecules of low molecular weight be administered by electrophoresis but also the active allergens of timothy grass, giant ragweed, and dwarf ragweed[3, 4] may be transported readily in this way into the skin of hypersensitive persons. In the continuance of previous investigations of the response of a small group to coseasonal therapy, seven patients were selected for preseasonal treatment by electrophoresis of giant and short ragweed extracts.

Because the electrophoretic administration of increasing quantities of a potent antigen is a new procedure, the treatment of hay fever by electrophoresis of antigen resolved itself into a problem primarily concerned with the development of a technique suitable for the administration of both giant and dwarf ragweed extracts in increasing dosage. This has been accomplished, and it forms, with the therapeutic results, the content of this communication.

UNITS OF DOSAGE

In order to have comparable results, certain variables necessarily encountered in electrophoretic therapy should be clearly defined. On the basis of these definitions an electrophoretic unit of therapy may be established.

From the Medical Service of Dr. George Baehr and the Laboratories of the Mount Sinai Hospital, New York, and the Biological Laboratory, Cold Spring Harbor, New York.

1. *Current.*—The current should be small enough to eliminate irritation due to the current itself. This depends upon the *current density* which is the ratio: $\frac{\text{current}}{\text{area}}$, or $\frac{\text{amperes}}{\text{cm.}^2}$. At the positive pole not more than 0.0005 ampere and at the negative pole not more than 0.0003 ampere of current density are employed. Since it is probably desirable to decrease the local skin reaction due to the introduction of antigen, the current density has been decreased in patients with hypersensitive (allergic) skins, to avoid severe reactions. The computed dosage of current, however, should be kept constant as the electrode area is increased.

2. Another way of administering approximately equivalent amounts with a low current density is to decrease the current density and to increase the time proportionately.

3. According to Abramson and Gorin,[2] the electrophoretic dosage, D, of ragweed extract should depend upon three factors which may be summated:

$$D = D_E + D_O + D_D.$$

D_E represents that quantity entering the skin by electrical mobility; D_O, the quantity entering by electro-osmosis; and D_D, quantity entering by diffusion. Although the importance of each of these factors depends upon the current and time, especially for very small currents and very short periods, this possible dependence may be neglected for the present. With this assumption, the electrophoretic unit of treatment by dialyzed extracts may now be defined.

4. Given a solution of dialyzed pollen extract *at a given pH and at a given concentration of total nitrogen,* an electrophoretic treatment unit may be defined in terms of current × time, independent of the area of the electrode. To ascertain a convenient standard unit, one must consider the quantities measured. If current is given in milliamperes and time in seconds, one milliampere for one second would be:

$$1 \times 1 \times 10^{-3} = 1 \times 10^{-3} \text{ coulombs} = 1 \text{ millicoulomb.}$$

The millicoulomb is the standard unit adopted here. That is, since current has the dimensions of $\frac{\text{coulombs}}{\text{sec.}}$, if current is multiplied by time, the dimensions of the proposed unit are:

$$\frac{\text{coulombs}}{\text{sec.}} \times \text{sec.} = \text{coulombs.}$$

A more natural unit might have a value of 1 coulomb or even 1 faraday. Thus, if a unit represented one milliampere flowing for 1,000 seconds, the value of the unit would be

$$1000 \times \frac{1 \times 10^{-3}}{1} = 1 \text{ coulomb.}$$

The millicoulomb is, however, more convenient.

METHODS*

Dialyzed giant and dwarf ragweed extracts containing 0.1 or 0.5 mg. of protein nitrogen per cubic centimeter were employed. The pH was adjusted, as previously described, to 7.4, and the solutions were preserved with merthiolate. A simple galvanic circuit was employed.

To test the sensitiveness of the patient, scratch tests were made with dilutions of the solution to be employed electrophoretically. The response to scratch testing indicated roughly what an initial electro-

*The ragweed employed was purchased from a commercial firm. The dried ragweed pollen was extracted in a Soxhlet apparatus with petroleum ether for several hours. Extracted ragweed pollen was then treated with distilled water (about 100 c.c. for each 10 Gm. of the pollen). The extract was dialyzed against cold, running tap water for several days and concentrated in vacuo. It should be emphasized that salt-free solutions are employed in treatment.

TABLE I

TYPICAL CHART OF PRESEASONAL TREATMENT OF HAY FEVER BY ELECTROPHORESIS IN 1939

DATE	GIANT RAGWEED					SHORT RAGWEED				
	MG. PROTEIN N PER C.C.	AREA IN SQ. CM.	CURRENT DENSITY IN MILLI-AMPERES	TIME IN MIN-UTES	REACTION	MG. PROTEIN N PER C.C.	AREA IN SQ. CM.	CURRENT DENSITY IN MILLI-AMPERES	TIME IN MIN-UTES	REACTION
5/10	0.1	1	0.5	5	Large confluent papules	0.1	2	0.25	5	Large confluent papules
5/17	0.5	1	0.5	1	Large wheal	0.5	2	0.25	1	Many scattered papules
5/24	0.5	1	0.5	1.5	Large wheal	0.5	2	0.25	1.5	Scattered papules
5/31	0.5	1	0.5	1.5	Large wheal	0.5	2	0.25	1.5	Scattered papules
6/7	0.5	1	0.5	1.5	Large wheal	0.5	2	0.25	2	Large number of scattered papules
6/14	0.5	1	0.5	1.5	Large wheal	0.5	2	0.25	2.5	Large number of papules
6/21	0.5	1	0.5	1.5	Large wheal	0.5	2	0.25	3	Large papules
6/28	0.5	1	0.5	1.5	Large wheal	0.5	2	0.25	3.5	Many large papules
7/5	0.5	1	0.5	1.5	Large wheal	0.5	2	0.25	4	Large papules
7/12	0.5	2	0.5	1.5	Large wheal	0.5	4	0.25	5	Large wheal
7/19	0.5	2	0.5	1.5	Large wheal	0.5	4	0.25	5	Large wheal
7/26	0.5	2	0.5	1.5	Large wheal	0.5	4	0.25	5	Large wheal
8/2	0.5	2	0.5	1.5	Large wheal	0.5	4	0.25	5	Large wheal
8/9	0.5	2	0.5	1.5	Large wheal	0.5	4	0.25	5	Large wheal
8/16	0.5	2	0.5	1.5	Large wheal	0.5	4	0.25	5	Large wheal
8/23	0.5	2	0.5	1.5	Large wheal	0.5	4	0.25	5	Large wheal
8/30	0.5	2	0.5	1.5	Large wheal	0.5	4	0.25	5	Large wheal
9/6	0.5	2	0.5	1.5	Large wheal	0.5	4	0.25	5	Large wheal
9/8	0.5	2	0.5	1.5	Large wheal	0.5	4	0.25	5	Large wheal
9/20	0.5	2	0.5	1.5	Large wheal	0.5	4	0.25	5	Large wheal

TABLE II

TYPICAL CHART OF PRESEASONAL TREATMENT OF HAY FEVER BY ELECTROPHORESIS IN 1939

DATE	GIANT RAGWEED					SHORT RAGWEED				
	MG. PROTEIN N PER C.C.	AREA IN SQ. CM.	CURRENT DENSITY IN MILLI-AMPERES	TIME IN MIN-UTES	REACTION	MG. PROTEIN N PER C.C.	AREA IN SQ. CM.	CURRENT DENSITY IN MILLI-AMPERES	TIME IN MIN-UTES	REACTION
5/22	0.1	1	0.5	5	No reaction	0.1	2	0.25	5	A few papules
5/31	0.5	1	0.5	1	No reaction	0.5	2	0.25	1	No reaction
6/7	0.5	1	0.5	2	No reaction	0.5	2	0.25	2	No reaction
6/14	0.5	1	0.5	3	No reaction	0.5	2	0.25	3	Very small papules
6/28	0.5	1	0.5	4	No reaction	0.5	2	0.25	4	No reaction
7/5	0.5	1	0.5	5	No reaction	0.5	2	0.25	5	No reaction
7/12	0.5	2	0.5	7	A few papules	0.5	4	0.25	7	Very flat wheal
7/19	0.5	2	0.5	8	No reaction	0.5	4	0.25	8	No reaction
7/26	0.5	2	0.5	10	No reaction	0.5	4	0.25	10	No reaction
8/2	0.5	4	0.5	6	No reaction; papules ½ hr. after leaving hospital	0.5	8	0.25	6	Papules
8/9	0.5	4	0.5	6	Very small papules	10.5	8	0.25	6	Very small papules
8/16	0.5	4	0.5	6	No reaction	0.5	8	0.25	6	Small papules
8/23	0.5	4	0.5	6	Very slight reaction	0.5	8	0.25	6	Very slight reaction
8/30	0.5	4	0.5	6	Papules	0.5	8	0.25	6	Papules
9/6	0.5	4	0.5	6	Papules	0.5	8	0.12	6	Papules
9/13	0.5	4	0.5	6	Papules	0.5	8	0.25	6	Papules
9/20	0.5	4	0.5	6	Papules	0.5	8	0.25	6	Papules

phoretic dose should be. As mentioned previously, constitutional reactions have been observed with the electrophoretic technique in an extremely sensitive patient. To illustrate the procedure employed in treatment, two tables are given in detail in which comparison may be made of a fairly skin-sensitive patient with another patient not as sensitive. It may be noted in Table I that the patient under consideration was given very gradually increasing doses because of severe local reactions. Large wheals continued to form during and following therapy by this method. This is also observed during the use of the injection technique. There was a more rapid increase in dosage in

the less sensitive patient described in Table II. The difference in skin reactions was marked. The control of the variables, however, is excellent with the electrophoretic technique because the level of the dose is always the same.

TABLE III

SUMMARY OF CASES

CASE	AGE	SEX	PREVIOUS TREATMENT	TOTAL ELECTROPHORETIC UNITS			RESULTS
				GIANT RAGWEED	DWARF RAGWEED	BOTH	
1	22	F	Coseasonal electrophoretic year before	540	620	1,160	Satisfactory
2	28	F	Coseasonal electrophoretic year before	1,320	1,380	2,700	Satisfactory
3	43	F	Coseasonal electrophoretic year before	1,650	1,650	3,300	Satisfactory
4	31	M	Dust during year	7,810	7,610	15,420	Satisfactory
5	43	F	Treated preseasonally by injection unsuccessfully 5 years before	1,330	3,870	5,200	Not satisfactory
6	34	F	Preseasonal injections year before	2,290	3,040	5,330	Satisfactory
7	18	F	None	5,310	5,250	10,560	Satisfactory

A summary of the cases reported here is given in Table III in which the number of electrophoretic units as defined by our method is described. Note that the number of electrophoretic units administered varied from 1,100 to more than 15,000. It seems likely that even greater dosage may be given by increasing the area of the electrode and the current.

Experiments to determine the exact quantity of protein nitrogen transported by electrophoresis into the skin have been begun. As yet we are not able to give the equivalent of the electrophoretic unit in terms of milligrams of nitrogen transported into the skin. However, since we do not yet know the nature of the active biologic constituents in the ragweed solutions used in therapy, we may, without hesitancy, increase the dosage by the electrophoretic method as carried out with the injection method even though the final purification of the material injected has not as yet been achieved.*

RESULTS

To evaluate the results of therapy by injections of ragweed antigen, even in a large series of cases, is, at best, difficult. In reporting the

*Recently the writer, in collaboration with Moore, Gettner, Gagarin, and Jennings, has isolated an unpigmented, highly skin-reactive substance from giant ragweed extract, using the Tiselius method of electrophoresis. This homogeneous material has been introduced into the skin by electrophoresis. Analyses of this fraction are in progress. (Abramson, H. A., Moore, D. H., Gettner, H., Gagarin, J., and Jennings, L.: Proc. Soc. Exper. Biol. & Med. 44: 311, 1940.)

series of seven cases enumerated in Table III, it should be emphasized that the satisfactory results of electrophoretic therapy are presented in Table I as a preliminary discussion, rather than as a representation of final claims.

When data were elicited for analysis, the patients were first asked to compare their symptoms of the season of 1938 with those of the season of 1939. The results of this comparison were so favorable to the electrophoretic method that they were immediately discarded. It was believed that education in avoidance of allergens, variation in pollen count, closer attention to local and nonspecific therapy, as well as electrophoretic vaccination itself, were playing significant roles. Instead of comparing the ragweed seasons of 1938 and 1939 directly in each case, another procedure was therefore followed. In the electrophoretically treated cases in which symptoms of hay fever occurred, the time of the onset of symptoms and the severity of the symptoms were compared with a much larger group of clinic and private patients treated by injection of ragweed extract. In general, the onset of symptoms toward the end of August occurred simultaneously in patients undergoing each type of treatment. Similarly, the absence of symptoms in early September was followed by more severe symptoms in certain cases during the third week in both instances. Moreover, there were no essential differences in the severity of symptoms. In other words, the electrophoretic and injection methods of vaccination as employed by the writer at present showed no essential differences in therapeutic results in the small series of cases reported. The electrophoretic technique deposits ragweed intradermally. It had been hoped that this intradermal administration of antigen, presumably so effective in other circumstances, might be equally successful with ragweed extract. However, there is an important difference in the response of the skin to ragweed in ragweed-hypersensitive persons. This difference is the whealing reaction. As shown by Abramson and Engel, wheal formation leads to an acceleration of absorption, even of large molecules.[5] Indeed, it was shown that colloids and whole blood disappeared much more rapidly in areas treated with histamine or at the sites of allergic wheals. Ordinarily, when antigens are injected intradermally, depots are formed which lead to antibody formation more effectively than a subcutaneous injection of the same antigen. In the case of ragweed electrophoresis, depot formation may be prevented by the ability of the whealing process to wash colloids out of the dermis.

It was found that epinephrine phosphate may be readily given by electrophoresis[6] to induce blanching. These blanched sites in certain instances were then used as the area for ragweed electrophoresis in patients with extremely hypersensitive skins in order to diminish the

local reaction. It may be that a combination of epinephrine blanching and electrophoretic vaccination of allergens would lead to better therapeutic results.

SUMMARY

The electrophoretic method of introducing certain allergens into the skin is a useful reproducible technique for transporting these allergens into the dermis.

Giant ragweed, dwarf ragweed, and timothy extracts have been employed in this way.

An electrophoretic unit of pollen dosage is defined.

Giant and dwarf ragweed extracts have been used satisfactorily in the preseasonal treatment of a series of seven cases of hay fever by electrophoresis of the skin-reactive constituents.

Analysis of undialyzed ragweed extract, using both the Philpot-Svensson and Longsworth techniques to estimate the amount of skin-reactive, unpigmented constituent present, has revealed that a very large amount of the unpigmented fraction is present in undialyzed extract of giant ragweed. We have,[7] therefore, been using undialyzed extracts in treatment experimentally since they are much simpler to prepare and less expensive.

It is possible to introduce large quantities of skin-reactive material from undialyzed extracts by electrophoresis. Several patients treated preseasonally in this way in New York City have thus far (Aug. 27, 1940) had a satisfactory season.

I am especially indebted to Mrs. Henriette Gettner for her assistance in these experiments.

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STUDIES WITH ANTIGENS

III. The Preparation of Purified House Dust Extract

C. H. Boatner, Ph.D., B. G. Efron, M.D., and R. I. Dorfman, Ph.D.
New Orleans, La.

I. fractionation with organic liquids and dialysis

THE purpose of this investigation was the preparation of a more highly purified and more potent extract of house dust than that obtainable by the methods in use previous to this work.

For the production of this purified extract, we have subjected aqueous extracts of house dust to fractionation by the addition of water-miscible organic liquids such as acetone, dioxane, and isopropyl alcohol. By varying the concentrations of these, the precipitation of various fractions of the original solute was effected.

The technique of the fractional precipitation may be described briefly as follows: The precipitate which formed when a small amount of the organic liquid was added to the original aqueous extract was removed, and then an additional amount of the organic liquid was added to the filtrate. This process was repeated to give progressively larger proportions of organic liquid, with successive separations of insoluble fractions. In this manner, the mixture of solids of the original house dust extract was separated into a number of fractions. The fractionations with organic liquids were carried out at low temperatures.

Fractional precipitation of the house dust extract with dioxane and other organic liquids resulted in its separation into three principal fractions, one only moderately soluble in water, the others completely so.

The results of comparative skin reactions showed that the allergic activity of the fractions precipitated by low concentrations of organic liquids was slight, that of fractions precipitated by intermediate concentrations was marked, and that of fractions precipitated by high concentrations, slight. The skin reacting activity of the fractions precipitated by the intermediate concentrations was found to be significantly greater per unit weight of extracted solids than that of the original extracts from which they were processed.

A single fractionation with any of the water-miscible organic liquids did not accomplish a perfectly clean-cut separation of inert from active constituents. Further purification was accomplished by a second refractionation. The skin reacting activity per unit weight of solid of fractionated and refractionated extracts was significantly greater than

From the Chemistry Department, Newcomb College, Tulane University, and the Allergy Division, Touro Infirmary, New Orleans.

TABLE I

CASE NO.	PERCENTAGE OF SOLIDS														
	3.5	3.15	2.8	2.45	2.1	1.75	1.4	1.05	0.87	0.7	0.52	0.35	0.18	0.11	0.07
1	Mm	Mm	Mm	m	Mm	Mm	Mm	m	ms						
	m	m	Mm	m	m	m	m	0	0						
2	M	Mm	Mm	M	m	m	Mm	m	s	m					
	m	m	ms	m	s	0	0	0	0	0					
3	Mm	m	m	m	m	Mm	Mm	m	Mm	m	s	s			
	ms	s	s	0	0	s	ms	s	s	0	0	0			
4	M	M	Mm	m	Mm	Mm	m	Mm	Mm	Mm	Mm	Mm	m	m	ms
	ms	m	m	ms	m	Mm	m	m	m	ms	ms	ms	s	s	0
5	M	M+	M+	M+	M+	M	M	M							
	m	m	M	m	Mm	Mm	m	m							
6	m	m	ms	ms	m	ms	s	s	Mm	Mm	Mm	Mm			
	s	s	0	0	s	0	0	0	s	s	s	0			
7	M	M	M	Mm	M	M+	M	Mm							
	m	m	m	m	m	Mm	m	m							
8	M	M	Mm	Mm	Mm	Mm	Mm								
	Mm	s	s	s	0	0	0								
9	Mm	m	m	m	m	m	m	ms							
	ms	s	0	0	0	0	0	0							
10	M+	M+	Mm	m	Mm	Mm	Mm								
	m	m	s	ms	s	0	0								
11	M	M	M	m	Mm	Mm	Mm	m							
	ms	ms	ms	m	ms	ms	ms	ms							
12	M	M	M	Mm	M	M	M	M							
	m	m	Mm	m	M	Mm	m	s							
13	Mm	Mm	m	m	m	m			s						
	s	s	s	0	0	0			0						
14	m	m	m	m	ms	ms			ms	s					
	s	s	s	s	s	0			0	0					
Controls (10 non-allergic patients)	0	0	0	0											
	0	0	0	0											
Eight allergic patients not sensitive to dust	0	0	0	0											
	0	0	0	0											

M + = very marked reaction; M = marked reaction; Mm = marked moderate reaction; m = moderate reaction; ms = moderate slight reaction; s = slight reaction; 0 = negative reaction. A description of the statistical analysis of comparative skin tests is being prepared at the present time.

The upper rows of symbols in each case indicate purified extract, the lower rows, original extract.

that of the original extracts. Still further refractionation of the highly purified fraction resulted in no further separation of inert material.

Dialysis experiments were instituted in which various semipermeable membranes were used. It was found that with No. 600 cellophane and with du Pont's tubular osmosis membrane, a considerable amount of active material dialyzed out along with inert solids. However, No. 1200 cellophane membrane was found to permit the passage of dialyzable material which contained very little skin reacting activity per unit weight of dissolved solids.

Table I shows the results of comparative skin tests of the purified extract and of the original extract from which it was processed. Analysis of the 127 observations shown in Table I gave the following results: The chi-square test gave the value of P to be less than 0.01. The purified extract produced larger reactions than the original extract 122 times; in four instances the sizes of skin reactions of the two were equal. Therefore, the skin reacting activity of the purified extract was significantly greater than that of the original extract. That this increased activity was due to an increased concentration of the amount of the specific allergic factor, and not to an increased concentration of the allergically inactive solids was evidenced by the following: Concentration of the dissolved solids of the two extracts was always equal; scratch tests performed on nonallergic persons failed to show any evidence of skin irritation when tests were made with both extracts; allergic persons who were not sensitive to house dust failed to show positive scratch tests with both extracts.

II. FRACTIONATION WITH SULFATE SALTS

Extracts purified by the above method were subjected to further treatment with ammonium, sodium, and zinc sulfate. This resulted in the formation of a fraction possessing a high degree of allergic activity. The three sulfates were found to be equally effective. Discussion, however, will be limited to a description of the use of ammonium sulfate for the preparation of the final purified extract.

An extract purified by fractionation and refractionation of original house dust extract with organic liquids was treated with ammonium sulfate by dissolving 60 Gm. of the salt in each 100 c.c. of the purified extract. A precipitate formed immediately. This was removed and then treated with a volume of water equal to that of the initial purified extract. The resulting solution was then cleared and again treated with ammonium sulfate as before. The precipitate which formed was filtered off and treated with a small volume of water, and the solution was dialyzed through No. 1200 cellophane against running water, until freed of sulfate.

Table II shows the results of comparative skin reactions with the purified extract obtained with organic liquids and dialysis, and of the fraction precipitated from this extract by ammonium sulfate.

Analysis of the seventy-seven comparative skin reactions shown in Table II, gave the following results: The chi-square test gave the value of P to be less than 0.01. The ammonium sulfate fraction produced larger reactions than the organic liquid fraction fifty-seven times; in twenty instances the sizes of the skin reactions of the two were equal. This indicates that ammonium sulfate treatment of an extract purified by fractionation with organic liquids produced further purification of the extract.

TABLE II

CASE NO.	PERCENTAGE OF SOLIDS							
	0.32	0.22	0.19	0.16	0.12	0.08	0.06	0.04
1	ms	ms	ms	ms	ms	ms	ms	s
	s	s	s	ms	s	s	0	0
2	M	M	M	M	M	Mm	Mm	m
	M	M	Mm	Mm	Mm	m	m	ms
3	Mm	Mm	Mm	Mm	Mm	m	m	m
	ms	ms	ms	m	m	ms	ms	ms
4	s	s						
	0	0						
5	m	m	m	m	ms	ms	ms	ms
	m	m	m	s	0	0	0	0
6	ms	m	m	s				
	0	0	0	0				
7	m	m	m	ms	s	s	s	
	m	m	s	0	0	0	0	
8	M	Mm	m	m	m	ms	ms	ms
	m	m	ms	ms	ms	ms	ms	ms
9	M	M	M	Mm	Mm	m	m	m
	m	m	ms	ms	s	0	0	0
10	M	Mm	Mm	ms	m	m	m	m
	ms	m	ms	s	s	0	0	0
11	M	Mm	Mm	Mm	ms	ms	ms	ms
	M	m	m	ms	ms	ms	ms	ms

Upper rows of symbols indicate ammonium sulfate fraction dialyzed; the lower rows show acetone-dioxane fraction dialyzed.

For explanation of symbols, see footnote to Table I.

It was then determined whether treatment of an original dust extract with organic liquids, ammonium sulfate, and dialysis produced greater purification than that which could be obtained by dialysis alone. The original unpurified extract was dialyzed through No. 1200 cellophane until 92 per cent of its total dissolved solids had been removed.

Analysis of 118 comparative skin reactions made with these two extracts gave the following results: The value of P was less than 0.01. The fully processed extract produced larger reactions seventy-six times and smaller reactions, three times, whereas the reaction sizes were equal thirty-nine times. From these data it was concluded that a greater degree of purification could be accomplished by fractionation

of original house dust extracts with organic liquids, ammonium sulfate, and dialysis than by means of extensive dialysis of original house dust extracts alone.

An original house dust extract was divided into two parts. One portion was fully processed as previously described. The second portion was precipitated and then reprecipitated with ammonium sulfate. Each was dialyzed at the same time, for the same period of time. Comparative skin tests showed that there was a significantly greater amount of activity per unit weight of solid in the fully processed extract than in the one treated only by ammonium sulfate and dialysis.

TABLE III

CASE NO.	PERCENTAGE OF SOLIDS												
	1.17	0.94	0.82	0.70	0.59	0.47	0.35	0.23	0.12	0.08	0.07	0.06	0.05
1	M	M	M	Mm	ms	m	m	s	ms	ms	ms	s	s
	s	s	s	0	0	0	0	0	0	0	0	0	0
2	M	M	M	Mm	m	m	m	m	ms	ms			
	Mm	ms	ms	s	s	0	0	0	0	0			
3	M	M	M	M	m	m	M	m	m	m			
	Mm	ms	ms	s	s	0	0	0	0	0			
4	M+	M+	M	M	Mm	M	M	m	ms	ms	ms	s	s
	s	s	s	s	s	0	0	0	0	0	0	0	0
5	ms	m	ms	ms	ms								
	s	s	0	0	0								
6	M+	M+	M	M	M	M	M	M	M	Mm	Mm	Mm	m
	ms	ms	ms	m	m	m	m	m	m	ms	ms	ms	s
7	M+	M+	M	M	M	M	M	M	M	Mm	Mm	M	M
	M	M	Mm	M	Mm	m	ms	s	s	s	0	m	0
8	Mm	Mm	m	m	m	m	s	s					
	ms	s	s	0	0	0	0	0					
9	M	Mm	Mm	M	M	M	m	m	ms				
	ms	ms	s	s	s	s	0	0	0				
10	M	Mm	m	Mm	Mm	Mm	m	m	ms				
	s	s	0	s	0	0	0	0	0				
11	m	m	m	m	m	m	m	m	ms				
	s	s	s	0	0	0	0	0	0				
12	M+	M	Mm	Mm	Mm	Mm	Mm	m	m				
	M	Mm	m	Mm	m	m	ms	s	s				
13	M	M	Mm	M	M	M	m	Mm	m				
	M	Mm	Mm	m	m	m	s	s	0				
Controls	0	0	0	0									
	0	0	0	0									

Upper rows of symbols indicate purified extract; the lower rows show original extract.
For explanation of symbols, see footnote to Table I.

Table III shows the results of comparative skin tests obtained with serial dilutions of the fully processed extract and those of the original extract from which the purified extract was processed.

Analysis of the 130 comparative skin tests showed the following results: The value of P was less than 0.01. In 128 instances the fully processed extract produced larger reactions; the sizes of the reactions were equal two times. In no instance was the size of the positive skin reaction with the fully processed extract smaller than with the original extract of the same solids concentration.

It was quite evident that the fully processed extract contained a greater degree of skin reacting activity per unit weight of dissolved solids than did the original extract from which the purified extract was processed.

SUMMARY

The processes of purification of house dust extract described resulted in the removal of a large proportion of allergically inert materials.

The purified and concentrated extract obtained by fractionation and refractionation of house dust extract with various organic solvents was further purified by dialysis through No. 1200 cellophane.

Extracts purified by fractional precipitation and by fractionation and dialysis contained a significantly greater degree of skin reacting activity per unit weight of dissolved solids than did the original extracts from which they were processed. Since the activity was significantly greater per unit weight of dissolved solids, it was evident that the increased activity of the purified extracts was due to an increased concentration of the allergic factor.

Precipitation of extracts purified by water-miscible organic liquids, with ammonium sulfate and other sulfate salts in high concentrations, produced further purification. This was significantly greater than that produced by treatment of the original extract with ammonium sulfate or by its extensive dialysis through a suitable semipermeable membrane.

Treatment of original house dust extracts or dialyzed original house dust extracts with water-miscible organic liquids removed nonspecific materials which were otherwise precipitated from these extracts by ammonium sulfate.

Treatment of aqueous extracts of house dust by fractionation with water-miscible organic liquids, and then treatment with ammonium sulfate or other sulfate salts in high concentrations, followed by dialysis, produced a highly purified house dust extract.

We wish to express our thanks to Margaret R. Pabst for her assistance in the computation of our statistics.

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THE FIRST REPORT ON HAY FEVER IN PALESTINE

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LITERATURE on the subject of pollen allergy in Palestine has never been encountered by the author. The majority of physicians whom I interrogated on pollen allergy in Palestine denied its occurrence entirely, while others, although agreeing that the disease is to be found, were of the opinion that it exists only in mild form and is never of long duration. Immigrants into the country were said never to suffer from the disease. My personal observations during the last four years have convinced me that the contrary is true.

I. GEOGRAPHIC AND CLIMATIC CONDITIONS

Although Palestine is a small country, it is varied in its geographic and climatic conditions. Palestine, according to the present political frontiers to the west of the Jordan River (i.e., without Transjordan), comprises a territory between 29° 30′ and 33° 15′ north latitude, bounded on the west by the eastern coast of the Mediterranean Sea and on the east by the Jordan River, the Sea of Galilee, and the Dead Sea. This small country of about 26,000 square kilometers is divided into three essentially individual climatic zones by mountain ranges stretching from north to south. These zones are:

1. The eastern chasm of the Jordan to the east, stretching along the Jordan River to the Dead Sea (400 meters below sea level). The climate is decidedly tropic in summer and very mild in winter.

2. The western coast and coastal plain with an equable (sea) climate of warm, humid summers and mild winters.

3. The zone of hills and valleys with altitudes up to 1,200 meters (Jerusalem, 800 meters), which lies between the first two zones. The climate of the hill zone represents a more continental type, with hot days and cool nights in the summer and cool winters.

Of the climatic features of Palestine, the most important is the fact that the period of rainfall is restricted entirely to the winter months between November and April. Occasional brief rains may also fall in October and May, but not in every year. A certain compensation for the lack of rain during the dry season is provided by the dew, especially rich in the coastal plain and the plain of Esdraelon.

Palestine is a pre-eminently sunny land, and in summer the abundant sunshine is burdensome rather than enjoyable. Because of the temperature, the growing period of plants sets in much earlier in the valleys and is retarded for a considerable time in the hills. The dif-

ference of from 1,000 to 1,400 meters in altitude is manifested in a difference of from four to eight weeks in the inception of the blossoming period, which occurs in the Jordan Valley from the end of February until the end of March, about one month, in the coastal plain from the beginning of March until the middle of April, i.e., from five to six weeks, and in the rest of the country from the end of March until the middle or end of May, or sometimes to the middle of June, i.e., from seven to eight weeks.

The absolute temperature peaks of the year do not occur in the summer, but rather in the spring (April to May) and autumn (September to October), and they are attended by the severe khamsins or siroccos. At these times the air becomes very dry (10 per cent relative humidity and even less), especially in the hill regions. This khamsin period is characterized by an extraordinary aridity and lack of wind, without the least drop in temperature during the night. The mucous membranes become excessively dry. In general, this period, which causes intense physical and nervous strain to almost everyone, is a critical ordeal for hay fever patients. Khamsins are responsible for general weakness and loss of resistance, and perhaps also for the consequent development of bronchial and asthmatic symptoms. Not an inconsiderable number of asthmatic conditions originate at such times.

II. THE CHIEF HAY FEVER PLANTS OF PALESTINE

Of the many wind-fertilized plants of Palestine, only those with practical significance are noted, and in addition, those which are not wind-pollinated, but which are deserving of special mention, owing to some particular individual importance.*

Ambrosia maritima, on the coastal plain, blooms in midsummer, from July until October.

Andropogon halepense and *hirtus* occur as weeds on irrigated lands throughout the country, especially from May to December.

Avena sterilis and *barbita* have their growth periods especially from March to May.

Chenopodium murale is present almost throughout the year.

Cynodon dactylon, or Bermuda grass, termed yablith or indgil in Palestine, is the most important hay fever excitant in the entire country, growing as a weed almost the year around; in some places it is planted for the sake of its decorative appearance in lawns.

Eragrostis cynosuroides, an autumn blossoming plant, occurs only in the coastal plain from Acre to Jaffa during the months from July to October.

Hordeum murinum, barley grass.

*I am especially indebted to Mr. Dinsmore of the American Colony, Jerusalem, and to Dr. Ibn Ari and Dr. Sohari, of the Department of Botany, Hebrew University, Jerusalem, for their helpful assistance with botanical suggestions and classification.

Hordeum vulgare, barley grass, which blooms in May.

Imperata cylindrica koenigii appears from May to June on the coastal plain and in the upper Jordan Valley.

Koeleria phleoides blossoms from March to April throughout the country, including the desert.

Mercurialis annua, the mercury plant, is spread throughout the country, but not very profusely, and it blooms from March to April.

Phleum tenue graecum occurs from March to April. (*Phleum pratense*, or timothy, does not occur here.)

Pinus halepensis, the Allepokiefer or Alleppo pine, blooms at the beginning of April and produces masses of pollen, but it does not come into question as a cause of hay fever.

Plantago psyllium, plantain, blooms from March to April.

Poa bulbosa, bulbons, meadow grass.

Poa annua grows in the hills and desert and blooms from March to April. *Poa pratensis*, the hay fever plant which grows in America, does not grow here.

Polygonum monopoliensis appears from March to April, especially in damp places.

Ricinus communis, castor-oil plant, occurs from March to August, especially in April and May. It grows only in the coastal zone, but it is widespread there.

Sorghum annuum is domestically cultivated in July, but only in Arab localities. (*Sorghum vulgare*, the chimney millet, durra, the main weed in Africa.)

Triticum vulgare, cereal wheat, grows throughout Palestine, except in the cities, and blooms from May to June.

Urtica urens and *U. pipulifera* appear from March to April.

Zea mays, corn, grows in the summer, but without great significance because of the weight of its pollen.

In addition to these wind-pollinated plants, a few others must be recorded because of their particular significance, especially several of the arboreous types. Of the greatest importance in this respect are the citrus species—orange, grapefruit, lemon, and mandarin, all of which bloom from April to May:

Citrus aurantium, orange and grapefruit.

Citrus limonum and *C. limonum dulcisa*, lemon and sweet lemon.

Citrus mandarensis, mandarin.

Others of this class are:

Calicotoma villosa, a legume, occurring especially in the hills in April.

Acacia retinoides, occurring from May to June.

Acacia longifolia, occurring from May to June.

Eucalyptus spp., blooming on the coastal plain from September to January.

Olea, the olive, blooming from the beginning to the end of May; although it is not a wind-borne pollen, the pollen is light enough to be grouped with the pollens of the wind-borne plants.

Robinia pseudoacacia, generally widespread, blooming at the end of April and May.

Other plants, such as rye, which are of importance in other countries, are not significant in Palestine.

The greatest importance attaches to *Cynodon dactylon*, the yablith or indgil. This grass, which grows in a wild state, as well as cultivated on lawns as the so-called "Egyptian indgil," may be compared in importance, because of the role it plays, with the North American ragweed. Thommen[2] describes the grass as follows: "Bermuda grass is more important than all the others combined, because its pollen is active, is produced in large quantities, is wind-borne and extremely buoyant, being about 20 microns in diameter. The plant is widely and abundantly distributed, growing on about 98 per cent of the lawns of the state." The irritative effect of its pollen is apparently very great. Up to the present, of all my hay fever patients in Palestine with more than two years' residence in this country, only two have failed to show positive reaction to extract of indgil. I have never encountered a similar sensitivity towards any one plant in Europe.

A special peculiarity of the country is the wide distribution of citrus plants. The "stupefying" odor of the blossoms in April and May in the vicinity of the orange groves, which may become diffused over many kilometers, occasionally forces even healthy persons to vacate the vicinity for a time, as they suffer from headache and nasal discharge. It is understandable that sufferers indicate the citrus blossoms as the primary cause of their hay fever. It would thus be possible for a person to speak, but quite mistakenly, of an "orange blossom" fever, making a false analogy to a "hay" fever. I have succeeded in demonstrating cutaneous allergy to orange pollen in only three patients to date, and then only in those who worked in orange groves. It was impossible to demonstrate an allergy to the odor (by filter) comparable to the sensitivity toward orange peel as manifested by definite allergic symptoms (rhinitis, eczema, asthma) and toward the volatile compounds of orange-peel oil. Allergy caused by these is found relatively frequently in Palestine.[3]

Distinction must be made in discussing the distribution of the disease between patients who are native-born inhabitants and those who are immigrants.

In examining the quantitative distribution of pollen allergy for the compilation of statistics, it is quite impossible to quote figures corre-

sponding, even to a small extent, to the actual incidence of the disease in the country.

Concerning the native-born population, hay fever unquestionably occurs among the Arabs, but no statistical data is available for a quantitative compilation of reports. Other allergic disorders, such as asthma, allergic eczema, etc., occur frequently among the Arabs. Native-born Jews seem to react with pollen allergy similarly to the European populations of Europe.

Immigrants offer some rather interesting material for study. They may be divided into four roughly delimited groups.

Group 1 consists of those who have already suffered from hay fever in America and Europe and who also had attacks during their first year of residence in this country. About 20 per cent of the patients fall into this class. In this group sensitivity is maintained toward the plants which grow both in America and Europe and in Palestine. It is noteworthy that, up to the present time, there has never been put on record a case of hay fever caused by *Ambrosia maritima*, which is widely distributed on the coast. Even Americans living here who were sensitive to the American ragweed remain unaffected by this weed.

Group 2 is made up of patients without hay fever for the first two or three years following their entrance into the country, but who then had symptoms. This is the largest group, comprising about 45 per cent. With the passage of time, sensitivity developed toward new, locally growing plants.

Group 3 consists of patients who remained free from symptoms for six, eight, or more years and then suddenly developed them from indgil, for example, upon its implantation in their vicinity for the first time. This group comprises about 10 per cent. Only a very few patients who were previously susceptible remained entirely unaffected (after more than ten years).

Group 4 is made up of immigrants, unaffected abroad, in whom symptoms developed for the first time in this country. They comprise approximately 25 per cent of all patients.

SUMMARY

The first report on the subject of hay fever in Palestine is submitted. Geographic and climatic peculiarities of the country are described.

The most important hay fever plants are enumerated, and their habitat and blossoming time are recorded. The hay fever evinced in this country is solely of the spring type, with the autumnal variety unknown. Of the hay fever-producing plants, the most important is *Cynodon dactylon*, or Bermuda grass, here known as yablith or indgil.

The distribution of hay fever among the Arab population is practically uninvestigated, although other allergic illnesses are frequent.

The non-Arab local populations are susceptible to hay fever in the same degree as Europeans. Of the immigrants suffering from the disease, 20 per cent comprise those who had previously suffered from the disease and were attacked in the first year of local residence; 45 per cent remained unaffected for two or three years and were then attacked; 10 per cent remained unaffected for six or more years before being attacked; and 25 per cent of patients of the immigrant group were attacked by the disease for the first time in Palestine, with no previous history of hay fever.

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Special Reports

TETANUS IN A SERUM-SENSITIVE PATIENT SUCCESSFULLY TREATED WITH A NEW DESPECIATED ANTISERUM

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THE administration of horse serum to highly sensitized patients has, in the past, been a tedious and rather hazardous procedure. Refinements are being made in the process of manufacture which tend to reduce the allergic reaction.

The elimination of the horse serum specificity has been accomplished by Coghill, Fell, Creighton, and Brown[1] by diluting the antitoxin (plasma) with distilled water containing phenol, and buffering this solution to a pH of 3.8, after which taka diastase is added and the mixture incubated at 37°. The solution is then filtered, and the filtrate is adjusted to a pH of 7.0. The antitoxic globulin is salted out, and the precipitated globulin is dialyzed and then diluted so as to contain the desired percentage of nitrogen. The antitoxin is finally made isotonic, filtered, and tested for despeciation by the direct anaphylaxis method in guinea pigs.

Scarlet fever, diphtheria, and tetanus antisera have been despeciated by this method. Scarlet fever antitoxin prepared in this manner has been used successfully by Toomey and Kimball,[2] with fewer allergic reactions. Baird[3] has had similar results with despeciated diphtheria and scarlet fever antitoxin. The successful use of tetanus antitoxin prepared by this method is exemplified in the following case of a patient who was extremely sensitive to the regular commercial tetanus antitoxin.

CASE REPORT

R. A. was admitted to the Receiving Hospital at 1:20 P.M. on July 29, 1938, complaining of stiffness of the neck and jaw, restlessness, and fever.

Present Illness.—On July 19, the patient received a dogbite on the left leg. The wound was not treated. On July 24, the right palm was lacerated by a rusty nail. The patient came to the Receiving Hospital for treatment immediately after the injury. The skin test with antitetanus horse serum was strongly positive, and the patient was told to return the next day for desensitization. He failed to return until July 29.

He stated that he felt well until July 28 when he became restless, chilly, and feverish. The next morning he developed headache and stiffness of the neck and

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jaw. There was no history of convulsions, loss of consciousness, diplopia, vertigo, tinnitus, dysphagia, or dyspnea.

Past History.—The patient had had diphtheria as a child, but he did not remember receiving diphtheria antitoxin. No other relevant history was obtained.

Physical Examination.—The patient was a well-developed and well-nourished white male of 22 years, who lay quietly in bed and spoke slowly with but little movement of the jaw. There was definite trismus and marked cervical rigidity, and the neck showed a positive Brudzinski sign. Swallowing was normal and there was no laryngospasm. The other muscles were flaccid. The tendon reflexes were normal; the abdominals were hyperactive, but equal. There were no sensory changes. Kernig's sign was negative. On the hypothenar eminence of the right palm there was a healing, jagged wound about 3 cm. in length, showing no evidence of infection and no spasm of nearby muscles. On the anterior aspect of the left leg, there were several linear lacerations. The surrounding area was tender, red, and swollen, but not fluctuant. There was no local spasm or twitching.

The palm laceration appeared clean. A necrotic area was found in the bottom of the leg wound. Both wounds were debrided. Open drainage was instituted, and wet dressings of 1:4,000 potassium permanganate were applied.

Toward evening on the day of admission, the patient developed marked irritability, opisthotonus, severe generalized muscular cramps, and laryngeal spasm, and he became irrational.

A diagnosis of tetanus was made. He was isolated in a quiet, dark room. A cradle was placed over the bed to avoid stimulation by bedclothes. Sodium phenobarbital and 25 per cent magnesium sulfate were given subcutaneously in doses sufficient to promote relaxation.

Because of the patient's sensitivity to horse serum, desensitization was attempted. One-tenth cubic centimeter of the regular commercial tetanus antitoxin was given intracutaneously. Immediately afterward, a large wheal with pseudopodia appeared, followed by an attack of bronchial asthma which required adrenalin. One-tenth cubic centimeter of the same horse serum was repeated one-half hour later, and again the patient developed a similar allergic reaction. It appeared, therefore, that this commercial tetanus antitoxin could not be administered safely. From a pharmaceutical house,* we obtained 300,000 units of a despeciated horse serum containing 2,000 units of tetanus antitoxin per cubic centimeter. The antitoxin titer of the new preparation was similar to that of the regular commercial serum. This new tetanus antitoxin was administered as follows:

7:00 P.M. (7/29/38)	0.1 c.c.	intracutaneously; no reaction.
7:45 P.M.	0.2 c.c.	subcutaneously; no reaction.
8:50 P.M.	0.4 c.c.	subcutaneously; no reaction.
9:20 P.M.	0.4 c.c.	intramuscularly; no reaction.
9:45 P.M.	0.8 c.c.	intramuscularly; no reaction.
10:30 P.M.	5.0 c.c.	intramuscularly; no reaction.
11:15 P.M.	1.0 c.c.	intravenously diluted in 250 c.c. normal saline; slight chill.
12:10 A.M. (7/30/38)	10.0 c.c.	intravenously diluted in 350 c.c. normal saline; very slight chill.
12:30 A.M.	10.0 c.c.,	5 c.c. each in and around the hand and leg wounds.
5:00 A.M.	10.0 c.c.	intravenously in 200 c.c. normal saline; very slight chill.
10:00 A.M.	10.0 c.c.	intravenously in 100 c.c. normal saline; slight chill.
7:00 P.M.	10.0 c.c.	intravenously, undiluted; slight chill.

Thereafter, 10 c.c. of undiluted serum were given every twelve hours intravenously until a total of 140 c.c. had been given. Only an occasional slight chill

*Parke, Davis & Co.

followed the remaining doses. By July 30, 1938, the opisthotonus and muscular cramps had disappeared, but the neck, jaw, abdomen, and extremities were still rigid. Temperature ranged between 102° and 103° F. until July 31, when it dropped to normal. On August 1, patient was flaccid, except for a mild trismus. This entirely disappeared by August 5. Both wounds healed well, and the patient was discharged on August 16. There was no evidence of serum sickness until August 24, when a mild urticaria developed.

This patient returned to the Receiving Hospital in July, 1939, because of acute gonorrheal urethritis associated with bronchitis. While here, he was retested with both the regular tetanus antitoxin and the new antitoxin. The reactions were as follows:

New tetanus antitoxin, 0.1 c.c. intradermally; wheal, 1.2 cm., and erythema, 3.0 cm.
Regular tetanus antitoxin, 0.1 c.c. intradermally; wheal, 1.8 cm., and erythema, 6.0 cm.

SUMMARY

The successful treatment of tetanus is reported in a patient who was markedly sensitive to the regular commercial tetanus antitoxin. The treatment was accomplished with a new equine tetanus antitoxin, despeciated by partial digestion with taka diastase. The mild degree of allergic reaction is especially noteworthy.

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A DEVICE FOR THE RAPID PERFORMANCE OF SKIN TESTS BY THE SCRATCH METHOD

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AN APPARATUS has been devised for the purpose of applying liquid allergenic extracts to the skin rapidly and in orderly sequence in a manner analogous to that used in printing with a rubber stamp. This device consists of three parts.

1. *Rack to Hold the Vials.*—This consists of a rectangular piece of wood, 13 by 8¾ inches and ¾ inch thick, perforated by four groups of holes, there being 24 holes to each group, 96 in the entire rack. The holes are 21/32 inch in diameter and are placed in rows one inch apart (from center to center), except at the margins of the groups where they are 1¼ inches from those of the adjacent group. Because of the fact that glass vials are not of uniform diameter and therefore do not fit snugly into these holes, a sheet of firm sponge rubber, ¼ inch thick, is cemented to the top surface of this board. The rubber is likewise perforated by the same number of holes and in the same locations, except that these holes in the rubber are only 9/16 inch in diameter. The openings on the bottom surface of the board are closed by a thin sheet of wood glued to its under surface.

2. *Vials.*—These are round glass applicator vials of ⅛ oz. capacity and approximately 19/32 inch in diameter. The bakelite caps, which contain solid glass applicator rods, were originally designed to screw on the vials. For the present purpose, however, the threads on the vials were removed with an emery wheel, thus permitting the caps to slip on and off easily.

3. *Rubber Stamp Applicator Device.*—A sheet of soft sponge rubber, 3¾ by 5¾ inches and ¾ inch thick, has a thin piece of wood cemented to its upper surface to provide rigidity. To its lower surface the tops of the bakelite caps are cemented in positions corresponding accurately to the locations of the vials in the rack. There are four such groups of 24 individual units to the entire rack of 96 vials.

OPERATION

Liquid extracts containing 50 per cent glycerine are placed in the vials to a level which is adjusted according to the size of the drop to be delivered by the applicators. (The higher the level, the deeper the applicators dip into the fluid, and, therefore, the larger the drops they deliver.) The size of the drops may be regulated further by lifting the applicators more rapidly or less rapidly out of the vials.

From the Departments of Medicine and Bacteriology of the University of Louisville School of Medicine.

The applicator device is removed from the rack and placed gently but firmly in contact with the skin so as to provide contact of all the applicators. Irregularities of the skin surface are compensated for by the sponge rubber. The application may be made to the back, arm, forearm, or elsewhere, with the patient in the upright or horizontal posture. The scratches are made through the drops after application.

ADVANTAGES

The chief advantage is the saving in time. Ninety-six extracts may be applied easily in less than one minute (without the scratches). Other advantages are the accuracy and uniformity of the applications, which are of value in preventing errors in the sequence of tests.

DISADVANTAGES

The chief disadvantage is the difficulty in performing tests with one or a few scattered extracts throughout the list.

It is believed that the advantages greatly outweigh the disadvantages and that the device adds much to the efficiency and economy of the office or clinic. We have used it in tests on more than 700 patients and find it to be of practical value.

Society Proceedings

THE AMERICAN ASSOCIATION FOR THE STUDY OF ALLERGY

ANNUAL MEETING, NEW YORK, N. Y., JUNE, 1940

The Use of Histaminase in Certain Allergic Conditions. L. E. PRICKMAN, M.D., Rochester, Minn. (Abstract.)

Our early experience with histaminase in the treatment of vasomotor rhinitis at the Mayo Clinic was difficult to interpret owing to the extreme variations in the material submitted for testing. Some batches gave excellent results; others, none. The product now being used produces much more uniform results. Clinical experience tends to indicate that histaminase is a valuable therapeutic agent in selected cases of vasomotor rhinitis and certain cases of physical allergy. The results are encouraging when histaminase is used both prophylactically and therapeutically in cases of serum sickness. It is also effective in selected cases of acute or recurring urticaria, particularly if food allergy is responsible. Certain persons allergically sensitive to certain foods apparently can tolerate these foods if prophylactic doses of histaminase are administered.

TABLE I

EFFECTS OF HISTAMINE THERAPY IN CERTAIN ALLERGIC CONDITIONS

CONDITIONS FOR WHICH T-360, T-360-K, T-360 KV, AND TORANTIL WERE GIVEN	RELIEF OBTAINED			
	DEFINITE		QUESTIONABLE	NONE
	MARKED	PARTIAL		
Vasomotor rhinitis*	32	7	7	9
Asthma		5	2	3
Urticaria	3		2	
Angioneurotic edema	4	1		
Allergy to foods	3		1	1
Migraine (allergic)			1	
Atopic dermatitis	1			
Sensitivity to liver extract administered parenterally	1			
Serum sickness (prophylaxis)	4	5		
Serum sickness (therapy)	9		1	2

*A number of the patients who had vasomotor rhinitis and who were relieved of their symptoms were patients who were clinically sensitive to foods, and their conditions might just as well have been classified as examples of allergy to food.

DISCUSSION

DR. FRANCIS M. RACKEMANN, Boston.—During this last winter, I took some time to review the literature on histaminase. There were several conclusions that seemed to me quite interesting. In the first place, there are a good many reports upon the clinical use of the substance, and

those reports vary widely. Most of them do not affirm the fact that it is useful, although there are two or three reports in which the results did seem to be pretty good. It is difficult to control those results. Just as Dr. Prickman showed, the patients in whom the substance seems to work well are also the patients in whom it is possible to show some change in environment, such as the removal of a dog, at the same time. It is hard to control the results.

When one considers laboratory experiments with histaminase, there is an interesting lack of correlation. In anaphylaxis in animals, apparently, the histaminase is capable of neutralizing the shocking dose very promptly. If histaminase is mixed with antigen in the test tube, neutralization takes place slowly, but, when it is injected into the animal before the shocking dose, neutralization is prompt. The time intervals are very different.

DR. HARRY S. BERNTON, Washington, D. C.—What method has Dr. Prickman used for determining the potency of this product?

DR. GEORGE PINESS, Los Angeles.—Dr. Prickman's timely paper is of interest to us because of the recent work with histaminase completed in our laboratories and clinic. We were unable to reproduce the work of Karady and Brown. From the laboratory animal experiments we were unable to get corresponding results. From the clinical side, the use of histaminase has failed, after critical clinical observations, to give the desired results that would justify considering this drug of value in the allergic diseases for which it has been recommended.

An analysis of Dr. Prickman's cases as reported today certainly does not convince one that they are relieved as a result of the histaminase.

Another interesting factor is the lack of stability of the drug, particularly during the period when it was placed in the hands of various workers for experimental use; this has been admitted by the manufacturers themselves who found it very difficult to prepare histaminase in a stable form. Our own experience proved marked deterioration of the product in a very short time. Assays indicate as much as 66 per cent deterioration.

We do not take the position that Dr. Prickman does, and in a recent article published in the *Journal of the American Medical Association* we reported our results which were practically 100 per cent failures. We are willing to stipulate that histaminase may have a place in serum disease, although that is questionable; and perhaps it may also be used for the headaches described by Horton. But in so far as the true allergic person is concerned, I have no hesitancy in saying that our results indicate the drug does not prevent allergic symptoms.

I hope that Dr. Prickman will continue his work with this substance and that the results of his work, our work, and the work of others will bring out the true value of the drug.

DR. JOSEPH HARKAVY, New York.—We have had occasion to study two groups of patients with cold allergy with histaminase. The patients of one group had Raynaud's disease, in which exposure to cold is followed,

as you know, with vascular spasm. The patients in the second group showed atopy with vasomotor rhinitis and, in addition, manifested symptoms of cold hypersensitiveness. In the group of patients with Raynaud's disease, absolutely no results were obtained following the use of large doses of torantil. In some of the atopic patients improvement of the cold allergy followed the administration of histaminase provided the patient abstained from the foods or inhalants to which he was sensitive. Histaminase alone while the patient came in contact with offending allergens was ineffective. In other words, when these patients were about 90 per cent improved as a result of the usual measures of immunization and elimination of foods to which they were sensitive, the superimposed cold allergy was controlled following administration of histaminase. In view of the small number of cases studied and the number of variables involved, it is impossible at this stage to draw adequate conclusions as to the real value of histaminase.

DR. ALEXANDER STERLING, Philadelphia.—Since the market has been flooded with histaminase, I decided to take a group of twelve patients with very severe urticaria and angioneurotic edema who have not responded to any form of treatment and to give histaminase a thorough trial. Not one patient has improved. One patient became much worse with histaminase. Adrenalin injections and care by a day and a night nurse were required to restore the patient to his original condition.

May I make a remark which may not be applicable to this particular paper? I think the way in which our Society is handling any new drug that comes on the market is a tragedy. I think we should do as other A. M. A. sections do and appoint a commission of reputable clinical allergists to whom all new therapeutic agents should be submitted first. Let them report the value of the medication before it is offered to the profession at large. Then the market will not be flooded with undesirable and useless drugs.

DR. H. OSGOOD, BUFFALO.—I want to ask whether Dr. Prickman has had experience with the parenteral use of histaminase, and, if he has, whether he has had any untoward reactions, due to the fact that it is derived mostly from pork.

DR. HARRY L. ALEXANDER, St. Louis.—I believe that, since the question of histaminase has been presented to this Society, many people are going to look to our action concerning the matter. No formal expression of opinion has been taken and no one really can know what we do think about it. Some of us believe it is good; some believe otherwise. Although no formal action should be taken, perhaps some statement should be formulated as to what our attitude may be toward this drug which certainly is popular at this time.

Perhaps my own feeling in the matter is not important, but I have been interested in its possibilities. Theoretically, if we have an enzyme that will effectively inactivate what we consider to be the substance released in the tissues that is responsible for allergic symptoms, it would be the long-awaited nonspecific means of treatment for which we have all been looking.

There are two things about histaminase which I believe have virtue. One is that it is an active enzyme which, in the test tube, will totally in-

activate histamine. Second, it is active in the intact animal. If one takes a rabbit, which has more histamine in its circulating blood than any other known domestic animal, one may completely inactivate all of it with histaminase. Unfortunately there has been no control work. I know of none anywhere in which this problem has been attacked constructively from the standpoint of dosage, method of administration, or just what allergic disorder it may be good for. We are dealing here with a lot of assembled reports with claims which are asserted and denied. So far, I feel that the clinical research on histaminase has not even begun. I know of no one as yet who is qualified to express an opinion on its clinical value that is supported by a sufficient number of cases treated with constant dosage and contrasted to adequate series of controls. Until such data appear, I suggest we hold our judgment in abeyance.

DR. PRICKMAN.—Dr. Bernton inquired about the potency of histaminase and how this is tested. As I have explained, at first the potency of the preparations varied greatly. At least one lot of histaminase was inert, but other lots were definitely active as far as their content of histaminase was concerned. Originally Code's method of testing was used, but now the histamine remaining in solution after incubation with histaminase is tested in the intact animal by observing its effect on blood pressure. The method is accurate, and the observations of the detoxifying power of histaminase in this material are convincing.

Dr. Piness referred to patients who were apparently benefited while taking histaminase in Rochester, but who failed to receive relief at home, and to one patient who had a relapse when a dog was brought back into the home. I emphasized that histaminase has not been as effective for patients who are sensitive to inhalants as for patients sensitive to foods or physical agents. I do not know the reason for this, but it may explain in part why some patients sensitive to inhalants have had recurrences of symptoms with a change of environment. It also may be the simple matter of using larger doses for patients sensitive to inhalants, but this is not yet clear. The group of patients Dr. Piness refers to are those who received questionable benefit from histaminase and who are not included in the group of forty-two patients with vasomotor rhinitis who received definite relief.

We have reason to believe that the product of histaminase my colleagues and I are now using is definitely stable; however, the early material did not remain potent over a long period of time.

I have had no experience in the treatment of Raynaud's disease with histaminase. However, if histamine plays a part in the production of Raynaud's disease (and I do not have any personal knowledge that it does or does not), relief from administration of adequate amounts of histaminase would be anticipated.

With regard to the question raised as to whether histaminase was marketed prematurely or before adequate clinical investigation had been carried out, I might say that clinical studies with histaminase have been carried out in this country since 1937, and, previous to that time, there had been a number of favorable reports in the European literature.

Dr. Osgood asks about the parenteral use of histaminase, and particularly whether we have encountered any trouble with patients who are sensitive to pork. So far, we have not had any reactions of this type, possibly because of changes which may occur during the preparation of the extract and also because it is not common to encounter patients sensitive to pork.

Migraine Reproduced by Injections of Specific Allergens. LEON UNGER, M.D., Chicago, Ill. (Abstract.)

I WAS fortunate enough to secure the cooperation of one migrainous person although she was aware that severe headaches would probably accompany the experiments. To this woman I offer my sincere thanks.

CASE REPORT

The patient, aged 49 years, whose mother and maternal grandmother are known to have had migraine, began to have migraine before the age of puberty. The attacks came on at irregular intervals and were not especially related to the menses. This patient came under my observation twenty-one years ago and has been in excellent physical condition, with the exception of the attacks of migraine. For approximately the first twelve years, the attacks were intermittent; almost all were very severe. Visual disturbances in the form of zig-zag lights and limitation of the visual fields were almost always present, and numbness occurred frequently. The headaches were usually unilateral, on one side or the other, but sometimes on both sides. The pain was frequently so severe that icecaps binding the head and sedatives gave little or no relief. Nausea and vomiting always occurred. The attacks rarely lasted less than a day or two; for a few days thereafter the patient felt weak. Between attacks there were no symptoms.

In 1931 cutaneous skin tests were done and were practically negative, but about this time urticaria followed the eating of cauliflower. It was soon found by clinical trials that the attacks of migraine were due to the ingestion of five foods: cauliflower, broccoli, and Brussels sprouts, which belong in one botanic family, and asparagus and curly endive. With avoidance of these foods, the migraine disappeared except for an occasional attack when the patient by error ate one of these five foods. She is so exquisitely hypersensitive that she has symptoms from traces of asparagus on a spoon. But, with these few exceptions, the patient had been completely free from symptoms for approximately nine years until the present investigations were started.

Before undertaking the experiments, the patient was examined thoroughly and found to be in excellent health. The urine and Wassermann tests were normal; blood counts were negative, with 4, 3, and 3 per cent eosinophiles on three counts. The fasting blood sugar was 83 mg., and the nonprotein nitrogen, 27 mg. per cent. The electrocardiogram was normal. Two basal metabolic determinations proved to be −8 and −16 per cent, respectively. Neurologic examination by a specialist in this field was made in April, 1940, and was completely negative. The fundi and visual fields were normal.

Cutaneous tests were practically negative, even to the five incriminated foods. Intradermal tests were positive for numerous foods, epidermals, house dust, silk, and kapok, but clinically the inhalants proved of no importance.

A systemic reaction in the form of migraine occurred a few hours after an intradermal test for cauliflower. Severe headache occurred, followed by nausea

and vomiting. The attack lasted over twenty-four hours and was not relieved by a hypnotic nor by amidopyrine. After an intradermal test for asparagus, the patient felt very tired, but migraine did not develop. Skin tests for Brussels sprouts and broccoli did not cause trouble even though clinically these have been shown to bring on migraine. To rule out a possible neurosis, diluting fluid deliberately mislabeled "cauliflower" was tested intradermally, with no symptoms.

Hyposensitization to the five foods was attempted last year. An extract was made from the five incriminated foods, and 0.10 c.c. of a 1:10,000 dilution of this mixed extract was injected subcutaneously as the initial dose. Vertigo, nausea, and vomiting occurred about twelve hours later, along with a mild headache. There was only a slight local reaction. One week later 0.02 c.c. of this same dilution (1:10,000) was injected intracutaneously. There was a considerable immediate local reaction, and again about twelve hours later, vertigo, nausea, and vomiting occurred. A month later the extract was diluted to 1:1,000,000, and 0.02 c.c. was injected with no local and no general reaction; since that time the dosage has been very gradually raised with this dilution without further trouble. Gradual increases will be attempted to see if the patient can be clinically hyposensitized by the procedure which has proved useful in other allergic conditions, e.g., bronchial asthma.

The leucocytic and clinical response to two of the five foods was ascertained, with the following results. After 8 oz. of cooked asparagus was eaten, the leucocyte count fell from a fasting 7,400 to 5,700, then rose to 6,200 and then fell to 4,600. There were intervals of twenty minutes between counts. One hour later the count fell to 4,500. This constitutes a very positive leucopenic index test. Ten hours after the test, vertigo appeared along with a mild headache. Marked nausea and vomiting occurred two hours later and lasted several hours. The patient felt weak and was sick for another twenty-four hours.

One month later 8 oz. of cooked cauliflower was given. This time the leucopenic index was negative as the white count jumped from a fasting 7,200 to 8,400 to 9,400, and then fell to 8,600. Interestingly enough, no symptoms occurred until thirty-one hours after the cauliflower was eaten. Then there occurred a very severe one-sided headache, associated with vertigo and followed by repeated nausea and vomiting.

Two hours after the headache began, 0.5 c.c. (0.25 mg.) ergotamine tartrate (gynergen) was injected subcutaneously and was repeated one hour later. Despite this, the headache lasted about twelve hours and was quite severe. Weakness persisted for another twenty-four hours. There was no variation in the percentage of eosinophiles after these two foods were eaten.

COMMENT

The case reported here adds one more link to the mounting evidence that true migraine is allergic. In this particular patient we were able to bring on migraine by feeding the responsible foods. This fact has been observed previously by many. But, for the first time, we have also induced attacks of migraine on three different occasions by deliberately injecting extracts of these foods. A search of the literature has revealed no previous report in which injections of a food extract have caused attacks of migraine.

In 1933, Rinkel[1] observed that the precipitation of migraine by various specific foods is not sufficient evidence to warrant the inclusion of

migraine in the category of allergic diseases. Rinkel's point is very debatable, for in our patient we were able not only to prove clinically that the migraine was due to eating the specific foods but we reproduced the headaches by injections of extracts made from these foods. Surely, no other proof is necessary to convince one that in this patient, at least, migraine is allergic, for we have carried out the same procedures which we all recognize as important in asthma, hay fever, and other allergic conditions.

In the treatment of inhalant migraine, Vaughan[2] reported that migraine was brought on by overdoses of house dust extract.

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DISCUSSION

DR. L. E. SEYLER, Dayton.—I agree with what Dr. Unger has said about many cases of migraine being allergic. Because the results of allergic treatment of migraine, in my hands at least, have not been nearly as successful as in some of the other types of allergic diseases, I have tried to find some method of investigating a case before beginning the allergic work-up, that will give an inkling of whether a good result may be secured. There has been some discussion about allergic headache as distinguished from true migraine. We all believe that we get better results in so-called allergic headache where there are nasal symptoms. I have often noticed in taking the history that a patient will have a true migraine history, with a story that he has a severe one-sided attack of headache that lasts eighteen to forty-eight or seventy-two hours and that he has nausea and perhaps vomiting; often, however, on being questioned carefully, he will also say that a few hours before his pain or nausea begins, he has a slight wetness in his nose and perhaps sneezing or blocking. In these cases I have had good results with allergic management. The patients who do not give a history of rhinorrhea during an attack are not nearly as likely to do well on allergic treatment.

DR. T. D. CUNNINGHAM, Denver.—My experience in migraine is similar to that of the other men, but I want to bring out one point. In women particularly, when an allergic history is present and no results are secured after removal of foods that correspond to positive skin tests, the menstrual history should be investigated. A great many will have a history of menstruating only one or two days, or three days possibly. With a little ovarian extract supplementing removal of foods, one gets beautiful results. Sometimes a similar result is obtained by anterior pituitary extract. With that combination, you clear up a good many of these migraine headaches when, otherwise, you would think the case a failure.

DR. WILLIAM C. SERVICE, Colorado Springs.—There is one point I would like to bring up and that is regarding the manifestation of mi-

graine in children. We so often fail to see typical migraine in the child because, as the pediatricians warn us, cyclic vomiting of childhood is the manifestation of migraine.

DR. CHARLES N. HENSEL, St. Paul.—In a group of patients with migraine Dr. Walter Boothby during the past two years has been using oxygen therapy by the Boothby mask. One of his valued laboratory technicians was a victim of recurrent attacks of migraine headaches which would occur five to six times a month. She had a prodromal aura of about three hours' duration. During the onset of one of these attacks of headache, she tried 100 per cent oxygen therapy by the Boothby mask for an hour and a half and the headache was aborted.

It was soon discovered that every time a headache was approaching, if she would take oxygen for one to two hours, the headache would not develop. If she delayed taking the oxygen until after the pain of the headache had actually appeared, oxygen therapy was of no avail, and the headache ran its usual course. So effective was the treatment that she had a tank of oxygen with a gauge and mask at home so that she could take oxygen day or night.

Dr. Boothby told me that over a period of nine months she had had only six headaches and that these were all mild in character, so that in this particular case it appeared that oxygen therapy decreased the severity of the attacks and increased the interval between the attacks. Boothby admittedly did not attempt to classify the headaches of these patients as allergic or nonallergic. He just referred to them as headaches and said that he had now collected about 100 cases where the application of 100 per cent oxygen during the stage of aura had been very efficacious.

In a recent conversation with him as to the mechanism by which oxygen therapy relieved headache, he referred to investigations done by some English authors who had injected diodrast intravenously in order to visualize the cerebral arteries during the prodromal stage of migraine. At that stage the arteries were found to be sharply and brilliantly outlined. But when the diodrast was injected after the headache had occurred, the outline of the cerebral arteries was hazy. Such an alteration was interpreted to mean that the cerebral arteries had gone into a state of spasm and had squeezed out serum into the perivascular space. Not until this extravasated serum was absorbed would the headache be relieved. In such a baffling malady as migraine, it is worth knowing that the administration of 100 per cent oxygen in the prodromal stage might be helpful.

DR. HAL M. DAVISON, Atlanta.—I want to ask the essayist what results he has obtained in treating headaches that are apparently migrainous but occur in connection with the menstrual cycle.

DR. UNGER.—Most doctors believe that migraine is incurable. Then ergotamine tartrate was introduced. Ergotamine tartrate has now been used effectively in such a large series of cases that there is no doubt whatsoever of its efficacy; but we do not want to permit the patient to have migraine and then relieve him by ergotamine tartrate. It is up to us to try to find out why the patient has migraine. I admit that the procedure takes a long time and requires every method at our disposal to come to a definite diagnosis as to the causative foods.

Answering Dr. Seyler, it is difficult to differentiate between the results in the nasal type and in the purely allergic migraine without nasal symptoms. We do not have enough cases. Those of the nasal type I have seen have been more benefited and have larger skin reactions. If a patient has a nasal blocking which leads to a frontal sinusitis and then headache, it is a question whether this is a true migraine or is merely a headache from frontal sinusitis.

As to Dr. Cunningham's remarks about gland extracts such as ovarian and anterior pituitary, those should be used where indicated. We have to use every means at our disposal. But we must not forget that the patient is allergic and that, if we can remove the foods to which he is sensitive, he probably will not need the gland extracts.

Migraine in childhood as shown in cyclic vomiting, as Dr. Service pointed out, is difficult to diagnose. One cannot predict that the child will develop migraine later on.

In answer to Dr. Davison, if the patient has headache only during the menstrual cycle, I would say that migraine is not present. In order to make a diagnosis of migraine, the patient must have symptoms at other times, between menstruations.

DEFINITIONS OF WORDS PERTAINING TO ALLERGY*

Adrenalin.—A trade preparation containing the active principle of the suprarenal gland; equivalent to epinephrine, q.v. This name is the first to be given to the substance and originated in 1901 with its discoverer, Takamine, then in the employ of the firm which continues to manufacture it. The name is banned by the Council on Pharmacy of the Medical Association, which substitutes the term "epinephrine."

Allergen.—Any agent capable of producing a state or manifestation of allergy. Adjective, allergenic.

Allergenic.—*See* Allergen.

Allergic.—Pertaining to, caused by, affected with, or of the nature of allergy.

Allergic coryza.—*See* Coryza, allergic.

Allergic dermatitis.—*See* Dermatitis, allergic.

Allergic rhinitis.—*See* Rhinitis, allergic.

Allergy.—Any specifically acquired alteration in the capacity of living tissue to react. This alteration in capacity to react results from exposure to an exciting agent and is manifested upon re-exposure to the same or to an immunologically related agent. According to some authorities, allergy may also be inherited.

That branch of medicine concerned with all specific acquired alterations in capacity to react.

Anaphylactic.—Pertaining to anaphylaxis.

Anaphylactogen.—A substance capable of producing a state or manifestation of anaphylaxis. Adjective, anaphylactogenic.

Anaphylactogenic.—*See* Anaphylactogen.

Anaphylactoid.—Resembling anaphylaxis.

Anaphylaxis.—A form of allergy occurring in certain species of animals. It is usually characterized by (1) prompt reaction upon exposure to the specific excitant, (2) serologic reactions of precipitation and complement fixation, (3) possibility of passive transfer, (4) Schultz-Dale reactions, and (5) other phenomena, which may vary with the species of the subject and with the nature of the allergen.

Anatoxin.—A toxin partly or wholly inactivated by formaldehyde and heat, yet remaining antigenic.

Anergy.—Absence of capacity to react (absolute anergy). A less than normal capacity to react to allergens or specific excitants; a capacity to resist the effects of potentially harmful agents, without clinical manifestations.

Angioneurotic edema.—A dermatosis characterized by acute transitory swellings of the skin and subcutaneous tissues and, often, the mucous membranes. The swellings are larger, deeper, and not as sharply defined as those of urticaria.

Antianaphylaxis.—A state in which the usual expected phenomena of anaphylaxis fail to appear. This refractory state usually occurs after a sensitized animal has been repeatedly exposed to a sublethal quantity of the respective anaphylactogen during the period of incubation.

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*Report of a Committee on Nomenclature prepared April 20, 1939, for the Association of Allergy Clinics of Greater New York by Dr. Wm. S. Thomas, chairman, Dr. Joseph Harkavy, and Dr. Samuel D. Bell, with the assistance of Dr. Marion Sulzberger.

Antibody.—A specific substance produced by living tissues upon exposure to an antigen and capable of reacting specifically with the same or related antigens. Among the antibodies are included alexins, amboceptors, agglutinins, antienzymes, antitoxins, bacteriolysins, cytotoxins, hemolysins, opsonins, precipitins, and reagins.

Antigen.—1. An agent capable of causing living tissues to produce antibodies. Among the antibodies are toxins, toxoids, ferments, precipitinogens, and various other allergens.

2. In common immunologic practice, an agent used in certain complement fixation tests, e.g., Wassermann antigen.

Areola.—An erythematous zone surrounding a papule, wheal, pustule, or other lesion of the skin.

Arthus phenomenon (reaction).—A specific local inflammatory reaction of the delayed type (24 to 48 hours) produced by direct introduction of an anaphylactogen into the sensitized tissues of an anaphylactic animal. It occurs in its typical form in the sensitized rabbit and is characterized by necrosis of the skin and subcutaneous tissue about the site of injection. It was first described by Maurice Arthus in 1921.

Asthma.—A syndrome characterized by dyspnea, either recurrent or continuous, with wheezing râles, a sense of suffocation, and usually cough. Asthma is usually a manifestation of hypersensitiveness to (1) inhaled substances, (2) ingested substances, or (3) bacteria of which the patient is a host.

Asthmatic bronchitis.—An inflammatory reaction in the bronchi due to infection, upon which an asthmatic state is imposed and which is caused by sensitization to the infecting organisms; or the asthmatic state may precede the bronchitis or occur simultaneously.

Atopen.—Any antigen or allergen (usually inhaled or ingested) which produces allergic manifestations of the atopic type.

Atopic.—Relating to, characterized by, or of the nature of atopy, q.v.

Atopic dermatitis.—An inflammatory dermatosis characterized by the distinctive phenomena of atopy. For description see the following titles in standard dermatologic texts: Neurodermatitis disseminata (Disseminate neurodermatitis), pruritus with lichenification, lichen simplex disseminatus, Besnier's prurigo, prurigo diathesique, prurigo mitis, prurigo temporania, and early and late exudative eczematoid.

Atopic reagin.—The antibody present in the serum of certain hypersensitive persons, through the agency of which the hypersensitiveness may be transferred to normal persons or tissues.

Atopy.—A peculiar form of allergy known to occur in man and characterized by:

1. Immediate vascular, exudative reaction upon exposure of the sensitive tissue to the specific excitant.

2. A familial tendency to acquire certain syndromes based on specific hypersensitiveness, e.g., asthma, hay fever, and atopic dermatitis.

3. The presence of certain antibodies usually designated as Prausnitz-Küstner antibodies or atopic reagins.

Autogenous.—Self-generated. As applied to microorganisms or their products, the term denotes those materials prepared from germs of which the patient is actually the host, as contrasted with stock material prepared from other sources.

Bacterial asthma.—Asthma due to bacterial infection.

Bacterial coryza.—Symptoms of a cold in the head due to hypersensitiveness to bacteria of which the patient is host.

Bronchial asthma.—Allergic asthma, as contrasted with other simulating forms of dyspnea.

Bronchoedema.—Swelling of the bronchial tubes, diminishing their lumina, hindering the passage of air through them, and causing dyspnea.

Bronchospasm.—Narrowing of the lumina of bronchial tubes by contraction of their muscular fibers.

Calmette test.—Tuberculin test made by instillation of a 1 per cent tuberculin preparation into the conjunctiva.

Cardiac asthma.—Paroxysmal or continuous dyspnea of asthmatic type due to cardiac failure.

Colloidoclasis.—(Colloidoclastic shock.) A breaking up of the physical equilibrium of the colloids in the living body, producing anaphylactic shock. It is attributed to entrance into the blood stream of unchanged (undigested) colloids.

Complement fixation test.—An immunologic test designed to ascertain the presence either of a particular antigen or antibody when the other is known to be present. Normal sera contain a thermolabile substance (viz., complement, alexin) which is essential to the completion of certain antigen-antibody reactions. The complement fixation reaction depends upon the fact that, under certain conditions, a measured amount of the said thermolabile substance is fixed (precipitated, deviated) only when both the antigen and its antibody enter into reaction with each other. Thus, in a system known to contain a particular antigen, the fixation of complement denotes the presence of the respective antibody, and vice versa.

Conjunctival test.—A test to determine allergic susceptibility of an individual. This test is usually carried out by the application of a few drops of pollen extract or of some dry pollen to the lower conjunctiva. It is commonly used as a supplementary method to determine pollen sensitivity in case of the absence of a positive skin reaction.

Constitutional reaction.—An immediate or delayed reaction following the administration of an allergen and occurring at sites other than that of its administration (local reaction) and other than that of the original symptoms (focal reaction).

Constitutional reactions are usually associated with local or focal reactions or both. For example, following injection of pollen extract, a hay fever patient may exhibit not only a local wheal and exacerbation of hay fever symptoms, but also generalized urticaria, dyspnea, gastrointestinal symptoms, and shock.

Contact dermatitis.—*See* Dermatitis, contact.

Coryza, allergic.—A cold in the head occurring in an allergic person and due to the influence of exposure to an agent to which he is hypersensitive.

Coseasonal.—During the season in which symptoms are present.

In treating pollinosis (q.v.), therapeutic injections of pollen extract begun or continued during the season of pollination of the respective plants are said to be coseasonal.

Cutaneous.—Pertaining to the skin.

Cutaneous reaction.—A response of the skin to a test of its capacity to react to a deliberate exposure to an excitant.

Cutaneous test.—Examination of the skin in respect of its capacity to react to a deliberate exposure to an excitant.

Cutireaction.—*See* Cutaneous reaction.

Cutis.—*See* Derma.

Delayed reaction.—An inflammatory lesion of the skin or other phenomena occurring hours or days after contact between allergen or atopen and hypersensitive

tissue cells. Such a reaction may be either local at the site of contact or systemic or constitutional.

Derma.—1. Cutis or true skin. The portion of the skin lying between the epidermis and subcutaneous fat.

2. A prefix signifying or pertaining to the skin.

Dermal reaction.—*See* Cutaneous reaction.

Dermal test.—*See* Cutaneous test.

Dermatitis, allergic.—Any inflammatory cutaneous condition due to the action of an allergen upon sensitized tissues.

Dermatitis, contact.—An inflammatory dermatosis usually resulting from external contact with a substance to which the patient is hypersensitive and characterized by pathologic changes situated principally in the epidermis.

Dermatophyte.—A microparasitic fungus (usually mold or yeast) growing in the human skin and setting up local lesions and often distant allergic manifestations, especially itching eruptions.

Dermatosis.—Any morbid affection of the skin.

Dermophyte.—*See* Dermatophyte.

Desensitize.—To destroy allergic hypersensitiveness due to an allergen or atopen. A procedure used in therapy of allergic manifestations. The method employed is usually that of parenteral injections of measured doses of the allergen itself.

Dick test.—An intracutaneous test devised by the Dicks of Chicago, to indicate the presence or absence of susceptibility to scarlet fever. It is performed with syringe and tubular needle. The test material consists of a filtrate derived from a broth culture of *Streptococcus scarlatinae*.

Distant reaction.—A specific response to an allergen occurring at a site remote from that of its introduction or application.

Domiciliary.—Pertaining to or carried on in the house, as domiciliary influences or agents.

Dust-proof.—A term applied particularly to fabric, textile, or other coverings for bedding, upholstery, etc., which are impermeable to dust particles.

Early reaction.—A response becoming clinically manifest and reaching its maximum within several minutes after sensitive tissue is exposed to an excitant. It usually refers to wheal and flare reactions, to scratch or intracutaneous tests, or to an anaphylactic or anaphylactoid reaction.

Emanation.—As used in allergy, a substance which is given off by a plant, living creature, or other object, especially the desquamation of particles of epidermis, dandruff, or hair or feathers from animals, birds, bedding, or articles of furniture or clothing.

Endermal.—In or into the true skin. A Greek derivative synonymous with the Latin one, intracutaneous.

Endogenous.—Generated from within. The term is applied to any exciting agent originating within a person's body, for example, asthma due to microorganisms of which the patient is the host.

Endotoxin.—A toxin generated and retained within the body of a microorganism.

Eosinophilia.—The presence in the blood or tissues of an unusually great number of eosinophile leucocytes.

Ephedrine.—A mydriatic alkaloid from mahuang *(Ephedra equisetina), Ephedra vulgaris* and other species of ephedra. It is related in chemical constitution and in physiologic action to epinephrine, being $C_6H_5CHOH.\ CH(CH_3)NH.CH_3$. It has an effect on smooth muscle, on secretion, and on the circulation similar to that of epinephrine, and is used in states of low blood pressure, shock, hemorrhage, and Addison's disease, and also in asthma and as a mydriatic.

Epinephrine.—An active sympatheticotonic principle derived from the medulla of the suprarenal endocrine gland. Levo-methylaminocethanol catechol, $C_9H_{13}O_3N_1$, is a synthetic preparation chemically identical with the epinephrine derived from the suprarenal capsule.

Ergin.—*See* Reagin.

Exogenous.—Having origin outside of the body; e.g., asthma due to inhalation of horse dander is said to be exogenous in origin as opposed to endogenous asthma caused by microorganisms of which the patient is the host.

Exotoxin.—A diffusible thermolabile poison produced by certain microorganisms having the property of causing primary tissue damage and of acting as antigens.

Extrinsic.—*See* Exogenous.

False-negative reaction.—An erroneous or deceptive negative reaction due to faulty technique or evaluation.

False-positive reaction.—An erroneous or deceptive positive reaction due to faulty technique or evaluation.

Focal reaction.—A response (exacerbation or recurrence) at the site of an active, quiescent, or healed lesion distant from the place of introduction or the point of origin of the exciting agent.

Focal symptom.—A symptom occurring at a given site.

Focus.—The primary source of infection that furnishes germs to whose products the patient is hypersensitive; e.g., an infected sinus causing a patient's asthma is said to be the focus.

Food asthma.—Asthma brought on by ingestion of certain foods.

Fungous.—Adjective proceeding from the noun fungus, q.v.

Fungus.—A cellular vegetable organism feeding on organic matter and reproducing by spores; included are mushrooms, molds, yeasts, and bacteria. *See* Dermatophyte.

Hay fever.—Allergic coryza due to pollen inhalation.

Hematogenous.—Produced by, derived from, carried by the blood.

Hypersensitive.—*See* Hypersensitiveness.

Hypersensitiveness.—A state or quality of more than normal sensitivity to a given exposure than is the norm, as determined by comparison with an adequate control group.

Hypodermatic.—Applied or administered beneath the skin, usually by means of a syringe and a fine, hollow, pointed needle.

Hypodermic.—*See* Hypodermatic.

Hypoergy.—*See* Hyposensitiveness.

Hyposensitiveness.—A state of less than normal capacity to react to a given exposure, as determined by comparison with a control group. Adjective, hyposensitive.

Hyposensitize.—To lessen an existing degree of sensitiveness.

Immediate reaction.—*See* Early reaction.

Immune.—Exempt or secure from the harmful effects of a pathogenic agent.

Immunity.—State of being immune. Specifically acquired resistance to a pathogenic agent.

Impermeable.—*See* Dust-proof.

Indirect test.—*See* Prausnitz-Küstner test.

Infection.—Invasion of the tissues by pathogenic organisms with resulting injurious phenomena.

Infectious.—Associated with or caused by living organisms. The original meaning of pestiferous or pestilential is seldom used now in allergic practice.

Infective.—*See* Infectious.

Inhalant.—An air-borne substance which is breathed into the respiratory tract; due to air-borne substances.

Inoculate.—1. To introduce vaccines or other antigenic material for preventive, curative, or experimental purposes.

2. To implant microbes or infectious material into or upon culture media.

3. To communicate a disease by transferring its virus.

Intracutaneous.—Within the true skin. In skin testing, the method of introducing material immediately beneath the epidermis by means of syringe and needle.

Intracutaneous test.—A trial or examination of the skin by means of the intracutaneous technique; *see* Intracutaneous.

Intradermal.—*See* Intracutaneous.

Intrinsic.—*See* Endogenous.

Irreversible.—Permanent, i.e., irreversible allergic reactions are those characterized by permanent pathologic changes in the parts affected.

Late reaction.—*See* Delayed reaction.

Leucopenic index.—The variation from normal of the leucocyte cell count of the blood. If ingestion of certain food is followed by a significant fall in the total leucocyte count, allergic hypersensitiveness to that food is believed by some workers to be present.

Local reaction.—The phenomena or lesions occurring at the site of application of an exciting agent.

Macule.—Minute area of skin, differing from the surrounding skin, visible but not palpable.

Mantoux (Mendel) test (reaction).—A test devised by Mantoux and performed by the intracutaneous injection of tuberculin. The characteristic reaction is of the delayed 24 to 48 hour type and indicates present or past infection with tubercle bacilli.

Mendel test (reaction).—*See* Mantoux (Mendel) test (reaction).

Microbic allergy.—Manifestations of hypersensitiveness due to contact with bacteria or molds, yeasts, etc.

Migraine.—Sick headache, hemicrania, megrim; a periodic symptom complex characterized by any or all of the following symptoms: pain in the head, usually unilateral, nausea, vomiting, photophobia, vertigo, and scintillating appearances of light.

Mold.—A minute parasitic or saprophytic fungus. When saprophytic, in some cases forming a wooly coating or mildew on the surface of its host and propagating by air-borne spores, it may act as a pathogen or allergen. When pathogenic, the vegetative hyphae may invade the host's cells.

Moro's test (reaction).—A skin test devised by Moro and performed by inunction of 50 per cent O. T., Koch, in an ointment base. A local erythematous reaction appearing in from 24 to 48 hours indicates present or past infection with tubercle bacilli.

Mould.—*See* Mold.

Mucous colitis.—A chronic or relapsing symptom complex sometimes due to hypersensitiveness to foods or bacteria and characterized by excessive mucus in the colon, and constipation or diarrhea, with colicky abdominal pain.

Naegele test (reaction).—A test described by Naegele of Berne, by Jaeger and by Tucker and Thomas in the United States, which consists essentially of the application of patch tests to lightly scarified or abraded skin areas, instead of to normal skin. This test often produces reactions when the patch test is negative, but the significance of these reactions requires further elucidation.

Neurodermatitis.—*See* Atopic dermatitis.

Nonallergic.—Not due to or not causing symptoms of hypersensitiveness. Not associated with hypersensitiveness.

Nonspecific protein.—An adjective phrase pertaining to the use of a protein, other than one causing symptoms. Referring to the use of a protein not related to the agent causative of any symptoms.

Nonspecific protein therapy.—Therapeutic use of any protein other than that to which the patient is allergic.

Normergy.—A normal degree of capacity to react.

Ophthalmic test.—*See* Calmette test.

Papular.—Pertaining to a papule, q.v.

Papule.—A small elevation in the skin or mucous membrane containing no free fluid and tending to persist for several hours or longer.

Passive transfer.—*See* Prausnitz-Küstner test (reaction).

Patch-abrasion test.—*See* Naegele test.

Patch test.—A skin test performed by applying a suspected substance to the surface of an apparently normal area of skin. The tested substance is placed in contact with the skin beneath an occlusive cover and retained for from several hours to days. In the specifically sensitive skin, a positive reaction usually appears within 12 to 48 hours in the form of an eczematous area.

Perennial.—Persisting throughout the year.

Perennial coryza.—Coryza which persists throughout the year, as distinguished from that which is present at certain seasons only, as during a pollinating time of the year.

Perennial treatment.—Treatment carried on throughout the year, as for seasonal hay fever, in contradistinction to preseasonal (q.v.) or coseasonal (q.v.) treatment.

Physical allergy.—Manifestations of allergy due to physical agents, such as cold, heat, wind, wet, etc.

Pirquet test (reaction).—A test to determine existence of past or present tuberculous infection in a patient, devised by Pirquet. The test is performed by placing O. T., Koch, in contact with a minute scratch, puncture, or abrasion through the epidermis. A positive erythematous reaction occurring in 12 to 48 hours usually indicates past or present infection with tubercle bacilli.

Pollen asthma.—Asthma occurring as a complication or manifestation of pollinosis or hay fever.

Pollen coryza.—Hay fever.

Pollinosis.—Allergic symptoms (usually hay fever) due to contact with pollens by a person hypersensitive thereto.

Prausnitz-Küstner test (reaction).—Local passive sensitization of human skin by intracutaneous injection of human serum containing a particular antibody. (*See* Atopic reagin.) The site so sensitized shows an immediate type of reaction (wheal and flare) when tested with the respective allergen.

Preseasonal treatment.—The prophylactic treatment by injection of pollen extract in a patient before the onset of hay fever.

Primary focus.—The seat of infection from which proceed (or which sets up) symptoms of disease elsewhere in the body.

Protein extract.—A protein substance dissolved out of materials such as foods or inhaled matter. Such extracts are used in testing and treatment of allergic patients who may be shown to be hypersensitive to them.

Pseudopod.—In allergic practice, an irregular projection of the margin of a wheal.

Quincke's edema.—*See* Angioneurotic edema.

Reaction.—The phenomenon resulting from the interaction of any two or more agents. A response to an excitant. In allergy, the phenomenon resulting

from the exposure of sensitized tissue to its respective allergen; e.g., a wheal following injection of pollen extract.

Reagin.—A specific antibody in the blood which, when injected into normal human skin or mucosa, makes the site specifically hypersensitive. Recently it has been shown that reagins may also sensitize certain animal tissues. *See also* Atopic reagin.

Recurrent reaction (local).—Reappearance or exacerbation of a local reaction.

Relief reaction.—*See* Therapeutic reaction.

Renal asthma.—Dyspnea occurring in connection with toxemia due to lack of normal elimination by the kidneys.

Renal dyspnea.—*See* Renal asthma.

Reversed Prausnitz-Küstner test (reaction).—An urticarial reaction appearing at an injection site when reagin-containing serum is injected into the skin of a person in whom the allergen is already present; the reaction which appears when Prausnitz-Küstner antibody is administered, not before, but after, the administration of the antigen.

Reversible.—Capable of changing from abnormal or pathogenic state back to normal condition.

Rhinitis, vasomotor.—*See* Coryza, allergic.

Sanarelli test (phenomenon).—A phenomenon believed by Sanarelli to show the presence of anaphylaxis in the intestinal epithelium (epithalaxis) and to be pathognomonic of human cholera. The phenomenon was produced by intravenous injection of a sublethal dose of live cholera vibrio followed twenty-four hours later by a similar injection of a filtrate of *Bacilli coli* or *B. proteus* culture. There followed hemorrhagic lesions in the small intestine, mesentery, and kidneys.

Schick test.—A skin test for determination of immunity from or susceptibility to diphtheria, as based upon the absence or presence in a person of substances specifically capable of neutralizing diphtheria toxin. The test is carried out by the intracutaneous injection of 0.2 c.c. of toxin solution containing $\frac{1}{40}$ of a single dose of toxin lethal for a 350 Gm. guinea pig. Absence of reaction indicates the presence of about $\frac{1}{30}$ unit of diphtheria antitoxin per cubic centimeter of blood and usually signifies clinical immunity.

Schultz-Dale test (reaction).—The specific production of contraction of an excised intestinal loop (Schultz) or of the excised (virginal) uterine strip (Dale) of the anaphylactic guinea pig, when the excised tissue is exposed to the anaphylactogen. The intestinal or uterine preparation must also be shown to be susceptible to specific desensitization.

Scratch test (reaction).—A skin test in which a scratch, an incision, or an abrasion is made through the epidermis, and the material to be tested is then placed upon the prepared site. The typical reaction is early and urticarial.

Sensitized vaccine.—A vaccine prepared from killed microorganisms, to which has been added material containing homologous antibody material.

Serum sickness.—A type of allergic manifestation following injection of a foreign serum and characterized by urticaria, fever, joint pains, and enlarged lymph nodes.

Shwartzman test (reaction).—*Shwartzman phenomenon (1928).* A phenomenon of local tissue reactivity in the skin and various organs to bacterial filtrates. Active principles responsible for the phenomenon were identified as antigenic soluble exotoxins. The phenomenon is produced by a preparatory (intradermal, intraparenchymal, or intravenous) injection of the active substance, followed by an intravenous injection of the active substance twenty-four hours later. Lesion produced at the site of preparation is severe hemorrhagic necrosis. (Shwartzman.)

Skin test (reaction).—A trial or examination performed by exposure of the skin to an excitant. Typical examples are (1) the scratch test, (2) the intracutaneous test, and (3) the patch test.

Specific protein.—A protein possessing capabilities of acting as an allergen.

Specific reaction.—In allergy the term is applied to phenomena produced by an agent which is identical with or immunologically related to one that previously produced alteration in capacity to react.

Status asthmaticus.—A condition of severe, long-continued asthma.

Stock vaccine.—A suspension of bacteria or virus obtained from a source other than the cells or secretions of the individual receiving it by inoculation or otherwise.

Subcutaneous.—Under or beneath the skin.

Subdermal.—*See* Subcutaneous.

Supersensitiveness.—*See* Hypersensitiveness.

Suprarenalin.—*See* Epinephrine.

Symptomatic reaction.—A reaction following testing or therapeutic injection of an allergen or atopen and characterized by the occurrence or reproduction of the original symptoms under investigation or treatment.

Systemic reaction.—*See* Constitutional reaction.

Test.—1. A trial or examination. 2. In allergy, a trial or examination pertaining to the allergic effects of a substance upon living tissues or fluids.

Therapeutic test (reaction).—Administration of an allergen or the employment of other specific procedures with the object of obtaining relief of symptoms and thus throwing light upon the possible nature of their cause.

Toxic.—Pertaining to, caused by, or of the nature of a poison.

Toxin.—Any poisonous substance of microbic, vegetable, or animal origin.

Toxoid.—A toxin which has been deprived of its poisonous quality, but remains antigenic.

Tuberculin.—A nontoxic extract of killed tubercle bacilli capable of producing specific reactions in tissues of infected human beings or animals previously infected with tubercle bacilli.

Tuberculin test (reaction).—A specific skin or other reaction to tuberculin. A specifically altered local or systemic reaction resulting from the introduction of an extract of tubercle bacilli into a human being or animal previously infected with tuberculosis.

Urticaria.—Hives; nettlerash. A dermatosis in which wheals are the distinctive and cardinal lesions, usually accompanied by itching.

Vaccine.—A preparation containing virus or microorganisms or their products, and used for purposes of diagnosis, for immunization, or other therapeutic effects. Vaccines may contain either living or killed organisms.

Vaccine, stock.—*See* Stock vaccine.

Vaccine test.—A skin test (q.v.) made with a suspension of killed bacteria in measured concentration.

Vasomotor rhinitis.—*See* Allergic coryza.

Vernal catarrh.—Conjunctivitis suspected to be of allergic origin, prevailing especially during the spring of the year.

Wheal.—A characteristic lesion consisting of a localized superficial swelling of the skin, often blanched, but sometimes without change of its color. The wheal reaches its full development and disappears within the period of from several minutes to several hours. Histologically the wheal consists of localized edema of the superficial cutis, slight dilation of blood vessels and lymphatics, perivascular infiltration, and increase in intra- and extravascular eosinophilic cells.

Wheal reaction.—The occurrence of a wheal (q.v.) at the site of a cutaneous test.

Selected Abstracts

Asthma and Hay Fever

UNDER THE DIRECTION OF SAMUEL M. FEINBERG, M.D., AND
THEODORE B. BERNSTEIN, M.D., CHICAGO

Humoral Antibody and Tissue Tolerance Induced in Pollen Sensitive Individuals by Specific Therapy: Preliminary Report. Loveless, M. H.: South. M. J. 33: 869, 1940.

Loveless reports the results of her continued work on the thermostabile circulating antibody that is produced in both ragweed pollen sensitive and nonallergic subjects as the result of repeated injections of ragweed pollen extract. A series of twenty-three ragweed-sensitive patients were given preseasonal therapy in 1939, consisting of weekly or semiweekly injections. Serum was prepared from the patients immediately prior to, during, and after the cessation of treatment. By means of the modified Prausnitz-Küstner technique described in an earlier publication, it was found that the serum of these patients exhibited an increased capacity to neutralize antigen, so that a mixture of stock serum (reagin) and heated treatment serum (thermostabile antibody) required as high as 1,000 units of antigen per cubic centimeter of serum for neutralization. In addition, the threshold of response of the skin or the conjunctiva to antigen was raised during treatment, so that some patients required from five to ten times as much extract to produce a skin reaction as large as before treatment, and 100 times more extract to give a threshold reaction in the conjunctiva. These data also tend to indicate that good clinical results were obtained in those patients who developed this increased tissue tolerance and thermostabile antibody as the result of treatment. In two patients on whom complete protocols were submitted it was shown that, after treatment had been discontinued, this antibody rapidly disappeared from the serum and the tolerance of the tissues to antigen was lost. The author suggests that the acquired immunity of the skin and conjunctiva is presumably due to the presence of thermostabile antibodies in the tissues and the inactivation of antigen by these antibodies would be expected to play an important part in preventing allergic manifestations.

Air-Borne Pollen in the Twin Cities Area With Reference to Hay Fever. Rosendahl, C. O., Ellis, R. V., and Dahl, O. A.: Minnesota Med. 23: 619, 1940.

The authors summarize under seventeen generalized group headings pollen data based upon the study of atmospheric slides exposed daily for four consecutive seasons in Minneapolis, Minn., from 1932 to 1935 inclusive. Pollen concentration figures are expressed in terms of pollen per square centimeter and pollen per cubic yard of air (Scheppegrell and Cooke methods). In the graphs are given data for each group, when each kind of pollen appeared and disappeared from the air, the time at which clinically significant concentrations of each kind of pollen are present, the period of highest pollen concentration for each group, and the amount observed. In some of the groups there was a uniformity of seasonal behavior noted; in others, variability was observed, which is of clinical importance. The authors point out the fallacy of

concluding clinical sensitivity upon the basis of a positive skin test only. They give figures in a group of 936 cases in which the percentage of patients with positive skin tests who were clinically sensitive varied from over 90 per cent in the ragweed, Russian thistle, sage, and grass groups to as low as approximately 10 per cent in some of the tree groups. It is important, therefore, to correlate the period of symptoms not only with a positive skin test, but also with data in regard to atmospheric pollen pollution.

Ragweed Situation in Eastern Canada in Relation to Hay Fever. Groh, H., and Minhall, W. H.: Canad. M. A. J. **43**: 258, 1940.

The Canadian Weed Survey of the Division of Botany and Plant Pathology has recorded since 1923 the occurrence of ragweed, along with that of all other weeds and poisonous plants in Canada wherever found. This paper is concerned with its distribution in Eastern Canada and is accompanied by a map upon which are recorded the findings in more than 2,800 local surveys. The peak incidence was found in the Montreal region and the area around the southern tip of Lake Ontario, and, in general, there was a well-maintained distribution along the lower lakes between these two areas. None of this borderland area of Ontario and Quebec has enough advantage over the prevailing conditions south of the border to commend it as a hay fever refuge. However, in general, territory to the north in Ontario and Quebec, as well as the vast area of northwestern Ontario and eastward to the Atlantic, is virtually though not entirely free of ragweed. In the Annapolis Valley in Nova Scotia is found the principal, but still moderate, heavy infestation in the east; however, in the same meridian to the north, the Gaspé peninsula is practically free of ragweed, and is being kept free of the plant by campaign and survey.

Effect of Aminophyllin, Histaminase and Nicotinic Acid on Histamin-Poisoned Puppy Bronchioles. Gilbert, A. J., and Goldman, F.: Proc. Soc. Exper. Biol. & Med. **44**: 458, 1940.

Investigations were conducted on the effect of aminophyllin, nicotinic acid, and histaminase upon puppy bronchiolar sections that had been previously constricted with histamine. Essentially, the method was to prepare thin sections of puppy lungs, which were mounted in a Petri dish containing Ringer's solution and the whole placed upon a microscope stage. The size of the normal bronchiolar lumen was measured; then an appropriate amount of histamine acid phosphate was added to the solution to constrict the lumen completely. Following this, the drug being investigated was added to the solution. It was found that aminophyllin caused a complete dilatation of the previously constricted bronchioles. Histaminase and nicotinic acid had little or no effect in the concentration used.

Fungi: Relation to Respiratory Allergy: Air Survey in Southern Michigan. Waldbott, G. L., Ascher, M. S., and Ackley, A. B.: J. Michigan M. Soc. **39**: 645, 1940.

A survey of air contamination in southern Michigan was carried on by the authors for a twelve-month period from November, 1937, to December, 1938. The culture plate method was used, consisting of identification and counting of colonies on Petri plates with Sabouraud's media incubated at room temperature after daily exposure for thirty minutes. The types of colonies found varied somewhat at different times of the year. *Penicillium*, yeast, and torula occurred throughout the year. *Alternaria* and *Monilia* were also present all year round, but showed a seasonal predominance

from May to November. Other fungi cultured were *Hormodendrum, Aspergillus, Mucor, Phoma, Rhizopus, Trichoderma, Trichothecium, Macrosporium, Fusarium,* and several other varieties.

The Incidence of Aspirin Hypersensitivity. Gardner, E., and Blanton, W. B.: Am. J. M. Sc. **200:** 390, 1940.

The writers attempted to determine by several methods the incidence of hypersensitiveness to aspirin. The records of 467 allergic patients, more than half of whom had asthma, were reviewed as to the use of aspirin and the effect noticed from its use. There were found five patients in whom aspirin was thought to influence the development of allergic symptoms. A group of fifty allergic patients were questioned in detail regarding the use of aspirin and quinine, and in none was a history of allergic symptoms provoked by their use elicited. Five grains of aspirin were given to 103 consecutive patients at the clinic (after a negative history of aspirin sensitivity had been obtained), and the patients were obtained for two hours. None of the 103 patients reacted violently to the drug, although two asthmatic patients complained of a feeling of slight chest constriction. Ninety-five allergists throughout the country were then circularized. Forty-six answered the questions regarding incidence of aspirin sensitivity. The average incidence based upon the answers received from this group was 0.2 per cent.

Dermatology

UNDER THE DIRECTION OF MARION SULZBERGER, M.D., NEW YORK, AND JOSEPH GOODMAN, M.D., BOSTON

Skin Hazards in American Industry. Part III. Schwartz, L.: Public Health Bulletin No. 249, Government Printing Office, Washington, D. C., 1939.

This bulletin represents the third of a series of studies of skin hazards in American industry. The volume consists of eleven contributions dealing with skin hazards in the citrus fruit industry, cigar manufacture, acid manufacture, pulp and paper manufacture, iron and steel manufacture, and the manufacture of organic solvents, of chromic acid and chromates, of glass, and of explosives. In addition, there are considerations of skin hazards caused by paints, varnishes, and lacquers, as well as skin hazards in photography, printing, and allied trades.

This bulletin and its two companions are indispensable for those interested in occupational dermatitis.

Sensitization to Picric Acid: Subsidiary Agents and Mode of Sensitization. Landsteiner, K., and DiSomma, A. A.: J. Exper. Med. **72:** 361, 1940.

Previously published studies have dealt with the correlation of sensitization phenomena with the relative lability of chemicals; the more labile the compound, the greater its sensitizing capacity. In this paper the authors have studies picric acid, a substance which is chemically similar to the nitro compounds previously studied, but in which the NO_2 groups are not readily replaced. It was chosen because it was known to produce clinical sensitivity in the human being.

In guinea pigs it was found that the application to the skin of dilute solutions of picric acid alone did not readily produce sensitivity. However, a more frequent sensitization was achieved by the use of butesin and picric acid (butesin picrate) or by the application of picric acid to an area which had been inflamed by the previous application of cantharides. A still greater degree of sensitization was achieved when butesin picrate was applied to the area previously treated with cantharides.

The authors think it likely that sensitization to picric acid is brought about by reduction of picric acid to form more reactive compounds. This mechanism differs from that previously studied (Cl and NO_2 substituted benzenes) in which conjugates were formed in the body by substitution for the Cl or NO_2 groups.

The Value of Liver Extract in Cases Intolerant to Arsenicals, Heavy Metals and Radiation. McKee, G. M., and Astrachan, G. D.: J. Invest. Dermat. **3**: 409, 1940.

The authors have studied the use of liver extract both for the prevention of intolerance and in the treatment of existing intolerance to arsenicals. Among their conclusions are the following: Liver extract is a useful therapeutic agent in some instances of intolerance, as well as a useful supportive measure for patients with a history of previous intolerance. Liver extract may have prophylactic value in patients who tend to develop pruritus, gastrointestinal disturbances, nephritis, pains in the bones and joints, and erythema with or without scaling. Liver extract is of no value in preventing nitritoid crises. In many instances injections of liver extract were followed by marked improvement in blood counts and decrease in icterus indices.

Purpura Hemorrhagica Due to the Arsphenamines: Sensitivity in Patients as Influenced by Vitamin C Therapy. Falconer, E. H., Epstein, N. N., and Mills, E. E.: Arch. Int. Med. **66**: 319, 1940.

Six patients sensitive to neoarsphenamine and one sensitive to bismarsen are reported in this study. Each patient repeatedly developed thrombocytopenic purpura after the administration of the drug to which he was sensitive. After saturation of these patients with vitamin C, no change in the sensitivity to the drugs could be demonstrated.

Immunology

UNDER THE DIRECTION OF MATTHEW WALZER, M.D., AND
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The Tissue Specificity of Brain and Medullated Nerves as Shown by Passive Anaphylaxis in Guinea Pigs. Bailey, G. H., and Gardner, R. E.: J. Exper. Med. **72**: 499, 1940.

Studies on the organ specificity of water-soluble brain substance are reported. Prolonged immunization of rabbits with a sedimented, heat-killed vaccine of *Pasteurella boviseptica*, grown in an infusion broth prepared from rat brain, yielded antisera containing antibodies for the broth as well as for the bacteria. Guinea pigs, passively sensitized with these rabbit sera, were shocked twenty-four hours later by the intravenous injection of autoclaved extracts of different organs of the rat. Similar extracts of the brains and other parts of the nervous system of several species of animals were also employed as antigens.

Severe or fatal anaphylaxis was obtained with extracts of rat brain and of a transplantable tumor of the rat. Very slight or negative reactions resulted with extracts of other rat tissues. The brain antigen was found to be organ-specific but not species-specific. It was present in the white matter of the central nervous system and in sciatic nerves, but was almost completely absent from the brains of fetal and newborn rats and rabbits. It was absent from the brain of the fetal guinea pig but was present soon after birth. The amount of the specific brain antigen in an animal seemed to be dependent upon the length of the period of gestation, the stage

of development at birth, and the degree of myelinization of the central nervous system of the animal. The anaphylactogen of brain broth was soluble in water, insoluble in strong ethyl alcohol, and thermostabile in neutral and slightly acid solutions.

Hypersensitivity in the Isolated Rabbit Heart Following Intrapericardial Sensitization. Seegal, B. C., and Wilcox, H. B., Jr.: Arch. Path. **30**: 416, 1940.

Rabbits were sensitized with either whole egg white or alum-precipitated egg white in one of three ways: (1) by a single intrapericardial injection of the antigen; (2) by a single intraperitoneal injection; or (3) by intravenous injection of the same quantity of antigen in ten divided daily doses. From four to seven weeks later, the anaphylactic reaction of the isolated perfused heart was studied by the method of Wilcox and Andrus.

The direct injection of egg white into the pericardial cavity produced sensitization of the heart in fourteen of fifteen animals. The injection of the same antigen by the intraperitoneal or intravenous routes resulted in sensitization of the heart in only seven of thirty-one animals. Hence, the direct exposure of the heart to antigen resulted in more effective sensitization of this organ. The results were the same irrespective of whether alum-precipitated antigen or whole egg white had been used for sensitization. The anaphylactic response in the perfused heart could be obtained at a time when little or no precipitin could be demonstrated in the circulation.

An Improved Serologic Method for the Determination of the Precipitative Titers of Antisera. Cannon, P. R., and Marshall, C. E.: J. Immunol. **38**: 365, 1940.

The serologic method now commonly used for the determination of the precipitin titer of an antiserum employs the serial dilution of antigen rather than of antibody and has been shown to be illogical and inaccurate. These writers have developed a relatively simple serologic method which determines the precipitative potency of an antiserum by dilution of the antiserum rather than of the antigen.

This method utilizes the combined methods of agglutination of collodion particles coated with a purified protein and the resuspension-agglutination technique. The method has been demonstrated to be sharply specific and delicate enough to demonstrate the presence of precipitins in animal sera so weak that no visible precipitate occurs when these are mixed with varying dilutions of specific antigen.

By this method, the authors have demonstrated specific precipitins in sera from patients hypersensitive to egg protein, tuberculin, and crystalline insulin.

Typical Anaphylaxis in the Dog in the Absence of the Liver. Waters, E. T., and Markowitz, J.: Am. J. Physiol. **130**: 379, 1940.

It has been generally accepted that the liver is essential for the production of typical canine anaphylaxis. The production of typical anaphylactic shock in dehepatized sensitized dogs is reported in the present communication.

Exclusion of the liver by ligation procedures is criticized on the grounds that the organ is not completely isolated from the circulation. Therefore, hepatectomy, under anesthesia, by the one-stage technique, was performed in dogs sensitized with alum-treated horse serum. Four of these animals, while still anesthetized, showed anaphylactic symptoms following the injection of the specific antigen. The criterion for anaphylactic shock was a pronounced fall in arterial blood pressure which immediately follows the shock dose of antigen. Changes in bladder tone were also noted in these animals. Administration of equivalent doses of serum in normal and nonsensitized eviscerated dogs under ether anesthesia had no effect on the arterial blood pressure.

The Elimination of Horse Serum Specificity From Antitoxins. Coghill, R. D., Fell, N., Creighton, M., and Brown, G.: J. Immunol. **39**: 207, 1940.

Experimental work is described which indicates that the digestion of horse antitoxic sera or plasma with the enzyme "Taka-diastase" yields a refined antitoxin from which the elements of species specificity have been eliminated almost entirely. Anaphylactic experiments in guinea pigs indicate, however, that traces of unaltered horse serum proteins may be present in the concentrated antitoxic preparations. According to the authors, the mechanism concerned in this change of a biologic specificity suggests that the process is not only one of purification, but also one in which the antitoxic molecule has been altered to a slight degree so as to change its biologic specificity.

The Placental Transmission of Antibodies in the Skin-Sensitive Type of Human Allergy. Sherman, W. B., Hampton, S. F., and Cooke, R. A.: J. Exper. Med. **72**: 611, 1940.

Quantitative studies on the placental transmission of skin-sensitizing and blocking antibodies are reported. Twelve sera from allergic pregnant women, drawn during the latter half of pregnancy, were studied. These contained skin-sensitizing antibodies to various antigens, such as pollens, danders, and wheat, in dilutions up to 1:1,000.

A comparison of these twelve maternal blood sera and the twelve corresponding cord sera by the method of passive transfer showed the human placenta to be impermeable to skin-sensitizing antibodies. Neither saline extract nor aqueous extract of fresh or frozen placenta showed evidence of the skin-sensitizing antibodies present in the maternal sera. Direct and passive transfer tests on six of these infants from 3 to 6 months of age were negative with antigens to which their respective mothers were sensitive.

The blocking antibody present in the sera of seven pollen-treated mothers with hay fever was also demonstrated in the cord sera. Further studies indicated that the blocking antibody disappeared from the infants' sera within from three to six months. The placental transmission of this antibody is further evidence that it is distinct from the skin-sensitizing antibody.

Two of six colostra showed a small amount of skin-sensitizing antibody, the titer of which was only from one-hundredth to one-thousandth that of the corresponding maternal sera.

Confirmatory evidence of the placental transmission of hemagglutinins and typhoid agglutinins is also presented.

Pediatrics

UNDER THE DIRECTION OF DR. WILLIAM C. DEAMER, M.D., SAN FRANCISCO

Anthropometry in Children. Progress in Allergic Children as Shown by Increments in Height, Weight, and Maturity. Cohen, M. B., Weller, R. R., and Cohen, S.: Am. J. Dis. Child. **60**: 1058, 1940.

Easily diagnosed and recognized allergic illness may be of less importance in an individual child's health than prolonged subclinical handicaps. In illustration are presented five case reports in which the progress of an allergic child from the standpoint of height, weight, and skeletal maturity is represented graphically. The authors find that when a disturbance begins, it first affects increment in weight. A more severe handicap disturbs increment in height, and lastly, progress in maturity is affected. Recovery occurs in the reverse order usually.

Failure to make up a deficiency, however, indicates marked and probably permanent damage. The degree of constitutional disturbance is a variable and individual matter, not necessarily related to the severity of other clinical signs. In general, constitutional disturbance is more apt to arise from gastrointestinal allergy than from respiratory allergy such as hay fever.

If other causes of growth pattern disturbance can be ruled out, the developmental assessment of a child can be used to determine the efficiency of the treatment of the allergy.

So-Called Epituberculosis. Sailer, S.: Am. J. Dis. Child. **60**: 900, 1940.

The thesis of this report is that "epituberculosis" is not an allergic reaction in previously sensitized tissue but an area of atelectasis secondary to compression on a bronchus by a tuberculous gland. In support of this concept Rössle emphasized the clinical observation that the epituberculous reaction did not always occur in the region of the primary tubercle. Morlock and Pinchin had also removed part of a hilar lymph node by bronchoscopy and witnessed the subsequent complete disappearance of a large shadow in the lung field immediately adjacent. Even the reappearance of "epituberculous" shadows after administration of tuberculin could well be explained by a corresponding change in the size of the compressing gland.

The author describes the case of a boy 7 years old in whom a large area of pulmonary atelectasis persisted for three years before it disappeared. Prominent lymph node shadows were present at the hilum of the involved side. The clinical symptoms were mild and the tuberculin test was markedly positive. The diaphragm was elevated and the rib interspaces were decreased on the side of the atelectasis.

Oral Desensitization in Food Allergy. Edwards, H. E.: Canad. M. A. J. **43**: 234, 1940.

Pointing out the fact that most authors discourage the attempt to desensitize to an offending food by the oral route, the author suggests that such an attitude is unwarranted. In common with Keston, Waters, and Hopkins, he finds oral desensitization to food to be simple, safe, and reasonably sure. He advises it where a food of importance in the diet is concerned. He recommends preceding the desensitization course with a two-week period in which the food is eliminated entirely. He warns against haste, recommends a fresh solution of the antigen daily, and warns against overdosage after the course of desensitization has been completed.

Twelve of thirteen attempts at desensitization were successful in the author's hands. Four of these attempts are reported in detail.

The Value of Elimination Diets in the Treatment of Infantile Eczema. Birt, A. R.: Canad. M. A. J. **43**: 520, 1940.

In a study of 198 cases over a ten-year period, a comparison is made of former and recent methods of handling infants with eczema. The difference consisted largely in the addition of elimination trial diets to the study of recent cases. Modifications of Cobb's diets were used. Egg was shown by this method to be the chief offender, with tomato, orange, milk, fish, oatmeal, and cod-liver oil following in the order named. Wool and soap were the commonest local irritants, while feathers, powder, and floor wax were also mentioned. The author feels that in addition to food, contact and environmental substances play an important part in the etiology of infantile eczema.

The results improved markedly when elimination diet trials were added to local measures, and the average period of treatment was reduced from 3.3 months to 2.8 months. An important advance was the removal of restraints as soon as possible in the hospital cases. This permitted itching to serve as a guide in the recognition of an offending dietary addition.

Skin tests were not done routinely. Twenty per cent of the cases lasted to or beyond the second birthday. A follow-up on forty-six of the patients five to ten years after infantile eczema showed that four had subsequently developed asthma; eight disseminated neurodermatitis; and ten, contact dermatitis.

Asthmatic Bronchitis Following Chronic Upper Respiratory Infection. Bivings, L.: J. A. M. A. **115**: 1434, 1940.

It is the author's conviction that chronic upper respiratory tract infection in children, occurring almost exclusively in winter and producing cough, malodorous breath, anorexia, loss of weight, recurrent colds, night sweats, and bronchitis, constitutes a definite disease entity. Although 35 per cent of such cases have asthmatic bronchitis, he feels the problem is not one of allergy but of infection. Eosinophilia and a family history of allergy are not usually present. Treatment, in addition to that which is local and supportive, includes the use of stock or autogenous vaccines. A case report of a 4-year-old girl with a history of repeated pneumonia and a family history of allergy, wheezing, and changing areas of lung infiltration accompanies the report.

Recurrent Vomiting in Children. The Factor of Food Hypersensitiveness. Fries, J. H., and Jennings, K. G.: J. Pediat. **17**: 458, 1940.

The etiology of a symptom complex in children, known as cyclic or recurrent vomiting, has been poorly understood. Metabolic disturbances, acute infections, organic disease of the gastrointestinal tract or central nervous system, and allergy have all been considered. Rapid dehydration, ketonuria, and a slight elevation of temperature are characteristic of the syndrome.

Six cases are cited in which recurrent episodes of vomiting occurred in children. In all, there was good evidence that specific foods were responsible, and in some, vomiting was intentionally induced by mixing the offending food with a barium meal. In all six instances some other allergic manifestation, such as eczema or asthma, had also occurred. The periodic nature of allergic manifestation and the tendency for food allergy to be masked by delayed onset or quantitative factors are cited as favoring the nonrecognition of an allergic etiology.

The presence or absence of ketonuria in the cases reported is not discussed.

Rhinology and Ophthalmology

UNDER THE DIRECTION OF FRENCH K. HANSEL, M.D., ST. LOUIS

A Case of Trichinosis Presenting Chemosis of the Bulbar Conjunctiva. Lahrfeld, L., and Breisacher, C. F.: J. A. M. A. **115**: 1794, 1940.

In view of a possible similarity of bulbar chemosis of this type and allergic reactions, it is important to differentiate the two conditions. The patient had an eosinophilia of about 25 per cent. Sections of biopsies taken from the gastrocnemius muscle showed well-developed cysts including the three-stage larvae of *Trichinella spiralis*. Examination of the eyes showed the lower bulbar conjunctiva to be intensely chemotic, so marked that it protruded between the lids. The conjunctival vessels were slightly injected and the entire conjunctiva had a yellowish, waxy appearance.

Conservative Treatment of Sinusitis in Children. Gewanter, R.: Arch. Otolaryng. **32**: 728, 1940.

In the conservative treatment of sinusitis in children, Gewanter emphasizes primarily establishment of an accurate diagnosis. He places a great deal of emphasis upon the occurrence of frequent colds. The commonest complaints of his patients were: (1) frequent colds; (2) nasal obstruction and discharge; (3) some degree of low-grade fever; and (4) cough. He calls attention to the importance of differentiating allergic complications. He uses the displacement type of therapy, employing 0.5 per cent solution of ephedrine sulfate in physiologic saline solution, or a 0.125 per cent solution of neosynephrine. By this method of treatment he reports early and satisfactory relief to children suffering from nasal sinus infections and cough.

Treatment of Sinusitis by the Displacement Method. Gundrum, L. K.: Laryngoscope **50**: 989, 1940.

Gundrum reports his observations on a group of 800 patients with sinusitis treated by the displacement method, using ephedrine, neosynephrine, bacterial antigens, and foreign proteins. By means of x-ray plates he has shown that allergic edema of the sinuses may be transitory. He emphasizes the importance of differentiating infectious sinusitis and allergy, and the recognition of possible combinations of these two diseases. He found that the displacement treatment with ephedrine or neosynephrine gave good results, but was much better if bacterial antigens were added. Among the 800 patients studied, the percentage of improvement with various medicants was as follows: (1) foreign proteins, 25 per cent; (2) ephedrine and neosynephrine, 58 per cent; (3) bacterial antigens, 71 per cent. Upon rechecking 100 patients by examinations and questionnaires from two to six years after they were discharged, the results appeared to be permanent in most cases.

Bronchostenosis Complicating Allergic and Infectious Asthma. Prickman, L. E., and Moersch, H. J.: Ann. Int. Med. **14**: 387, 1940.

According to the observations of Prickman and Moersch, among 140 asthmatic patients examined with a bronchoscope, sixty were found to have definite stenosis of one or more bronchi. They state that bronchostenosis is primarily inflammatory and not referable to allergic edema or bronchial spasm. A study of these patients showed that bronchostenosis complicating asthma generally produces characteristic symptoms and frequent physical and x-ray signs. One prominent symptom is severe, persistent, and sometimes paroxysmal cough. At first it is impossible for the patient to raise sputum, and when it appears, it is profuse, usually mucopurulent, and sometimes streaked with blood. Febrile episodes either with or without preceding chills occurred in 68 per cent of the cases. Of the sixty patients, 53 per cent gave a history of pneumonia, and 35 per cent, of pleurisy. After the acute phase subsides, bronchoscopic examination reveals a stenosed bronchus; dilatation by forceps introduced through the bronchoscope and aspiration of the retained secretion usually relieve it. Other physical signs are secondary to the atelectasis. The authors state that almost every case of bronchostenosis complicating asthma may be traced to an acute infection of the respiratory tract.

Correspondence

The Editor,
THE JOURNAL OF ALLERGY

In THE JOURNAL OF ALLERGY, November, 1940, there was published an article by Dr. Morris Kalmon, "A Clinical Comparison of Epinephrine in Oil and Epinephrine in Gelatin." He states: "The oily preparation apparently is not best suited for use by the patient at home since a dry sterile syringe is advocated and this is very inconvenient for a lay person to attain." I should like to correct the misconception which pertains to the necessity of a dry sterilized syringe for the administration of epinephrine in oil. In the December, 1939, issue of the *American Journal of the Medical Sciences*, I published an article entitled "Epinephrine in Oil. Its Effectiveness in the Symptomatic Treatment of Bronchial Asthma." Because this article has apparently escaped the attention of many investigators, as well as one of our large drug houses, I wish to call to your attention the second paragraph:

"A change has been made in the technique of administration. It was formerly suggested that dry sterilized syringes and needles should be used. The purpose of this was to conserve material put up in multiple dose packages by preventing contamination, and, therefore, oxidation of the epinephrine by water introduced from a syringe that had been sterilized by boiling. The epinephrine in oil is now prepared in 1-c.c. ampoules and the annoying technique of dry sterilizing may be abandoned. Epinephrine in oil is not harmed by the meager and transient contact with water in a syringe that has been boiled."

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